STUDIES ON THE REPRODUCTION OF INDIAN WHITING *SILLAGO SIHAMA* (FORSKAL) (PERCOIDEI, SILLAGINIDAE)

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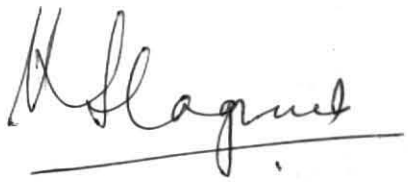
JUNE 1989

CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON THE REPRODUCTION OF INDIAN WHITING *SILLAGO SIHAMA* (FORSKAL) (PERCOIDEI, SILLAGINIDAE)" is the bonafide record of the work carried out by Shri. P. Jayasankar under my guidance and supervision and that no part thereof has been presented for the award of any other degree.



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DECLARATION

I hereby declare that this thesis entitled "STUDIES ON THE REPRODUCTION OF INDIAN WHITING *SILLAGO SIHAMA* (FORSKAL) (PERCOIDEI, SILLAGINIDAE)" has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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CONTENTS

PREFACE

From the point of view of rational exploitation and proper management of the fishery resources as well as for the development of intensive aquaculture of fishes through selective breeding, brood stock development, domestication and genetic improvement, a sound knowledge of reproductive biology and physiology of the candidate species is of great importance. In recent times, a wealth of information on maturity, spawning habits, spawning periodicity, spawning season, size at maturity and fecundity of commercially important fishes has been generated.

Gametogenesis involves the transformation of Primordial germ cells in the gonads into specialised cells or gametes, namely ova in the female and sperms in male, through a series of complex morphological and cytological events. The formation of male gamete is known as spermatogenesis. In the female, the primary growth phase involving the formation of primary oocyte from oogonia is known as oogenesis, which would be followed by the secondary growth phase, in which considerable increase in the size of the oocyte occurs, due mainly to accumulation of yolk. This process is known as vitellogenesis, which would be followed by final maturation and ovulation of the ova.

In the present work, basic aspects of maturation and spawning, salient features of gametogenesis and associated biochemical changes occurring during these processes in an important cultivable fish, *Sillago sihama* belonging to the family Sillaginidae have been investigated.

The fishes belonging to the family Sillaginidae, commonly known as 'sand whitings' or 'sand borers', are distributed in the shallow estuarine and near coastal waters of Indo-West Pacific region. Few species are also known to ascend the freshwater regimes of the river. The sillaginids contribute to small but commercially important fisheries throughout the range of their distribution (Mckay, 1985).

The sillaginids are gaining importance in fish farming. At present sillaginid culture is in its beginning stage and restricted to few countries. But they have great potential for augmenting fish production through aquaculture.

Characteristics such as capacity to tolerate wide fluctuations in environmental conditions, fast growth rate and above all their great demand as a delicious table fish, make sillaginids favourite candidate species for culture in coastal sea water, estuaries and brackishwaters.

In India, the sand whitings are caught all along the coast in nearshore seas, estuaries and brackish water lakes. Some of the important fishing areas in the country are Chilka lake, Kakinada, Vishakhapatnam, Pulicat lake, Palk Bay, Gulf of Mannar, backwaters of Kerala, Karwar, Netravathy and Gangolly estuaries..

Among the different species of Sillaginids occurring in the Indo-West Pacific region, *Sillago sihama* is the most widely distributed species. In Indian waters, studies on the seed availability of this species have indicated that large numbers of fry and fingerlings could be collected from the inshore waters, estuaries and backwaters almost throughout the year. This offers immense propsects for large scale culture of the

species in the country. Preliminary experiments of the culture of *S. sihama* have been carried out at Mandapam and Mangalore.

Some information is available on the biology, breeding, larval rearing and tank culture of Sillaginids. However, the taxonomic status of the family Sillaginidae has been in a confused state and a comprehensive knowledge on maturation, spawning and gametogenesis of *S.sihama* is lacking. In view of these lacunae investigations on different aspects of reproduction in this species along with a systematic study of the sillaginids from the area of present work were taken up and the results are presented in this thesis.

The thesis is presented in 8 chapters. Chapter-I surveys the literature on distribution, fishery, taxonomy, biology and culture aspects of sand borers of the Indo-West Pacific region. Various methodologies employed in the present study are mentioned in Chapter-II, followed by taxonomic consideration of the whiting species from Palk Bay and Gulf of Mannar. In Chapter-IV, general aspects of reproductive biology, such as external morphology of gonads, classification of maturity stages, spawning season, gonado-somatic index, size at maturity, fecundity and sex-ratio are considered. In Chapter-V, histological structure of gonads and the cellular changes occurring during oocyte growth and spermatogenesis are discussed. Chapter-VI deals with the biochemical changes occurring in certain somatic tissues and the gonads with respect to maturation. Results of the histochemical changes taking place in the oocytes during maturation are discussed in Chapter-VII. Finally in Chapter-VIII, results of some preliminary experiments carried out on induced maturation and spawning of the species are presented.

The study reveals that 6 species of fishes belonging to the family Sillaginidae occur in the Palk Bay and Gulf of Mannar, among which *Sillago sihama* accounts for about 60 percent of all the species in the commercial landings at Mandapam and surrounding areas. The other species in the order of abundance are *Sillago indica, S. argentifasciata, S. soringa, S. vincenti and S. chondropus*. It is found that *S. sihama* is a prolonged breeder whose spawning season extends from July to February. The size at first maturity of the female is slightly more than that of male. *S. sihama* is a high fecund fish, whose fecundity is curvilinearly related to length and linearly related to both body weight and ovary weight. Month-wise sex-ratio is not significantly different from 1:1 ratio, though females out-number males significantly above 170 mm (total length) in the commercial catches. The ovary is of the cystovarian type and the testis lobular type with unrestricted distribution of spermatogenic cells. In the oocytes, thecal layer is poorly defined and the zona radiata has a bipartite structure. Zona radiata interna shows undulated nature in the secondary and tertiary yolk granule oocytes, which is indicative of the micropinocytotic activity in connection with vitellogenesis. Yolk mass formation is of the 'continuous' type. Lamp-brush chromosomes are detected in the nucleus of Vacuolated oocyte stage which disappear as the maturation of oocytes proceed. Nuclear membrane exhibits 'bleb' formation in the Primary yolk granule oocyte stage. Four stages of atresia are recognized in the vitellogenic follicles. Yolk granule oocytes and hyaline oocytes are relatively more important than the other oocyte stages in the mature and ripe ovaries respectively.

During maturation, carbohydrates, protein and lipid from the body get translocated to the gonads for the formation of gametes and hormones. Carbohydrates are detected richly in the zona radiata externa and yolk vesicles of the oocytes. Lipid yolk makes its appearance first, followed by yolk vesicles and protein yolk almost simultaneously. DNA content is detectable only in the nucleus of pre-vitellogenic oocytes. 'Nucleolar extrusions' are observed. With carp pituitary gland extract and HCG, the species could be made to spawn in the laboratory; other details of the results are discussed. These are some of the original contributions of the present investigation. The results of the work have cleared the confusion about the taxonomy of whitings from Palk Bay and Gulf of Mannar, contributed new information about the finer aspects of reproduction in *Sillago sihama* and showed that this species could be induced to spawn in the laboratory, which forms the initial step for the development of its artificial propagation programme for intensive aquaculture.

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CHAPTER - I

INTRODUCTION

The sand whitings or sand borers of the family Sillaginidae are highly esteemed food fishes occurring in the inshore and estuarine waters of the Indian and Western Pacific oceans from South Africa to northern Japan. They occur in eastern parts of Africa, north-west Madagascar, Red sea, Persian gulf, Gulf of Oman, Seychelles, east and west coasts of India, Sri Lanka, Burma, Sumatra, Philippines, Singapore, Thailand, Indonesia, Taiwan, China, Borneo, Japan, Korea, New Guinea, Solomon islands and Australia (Mckay, 1985). Maximum number of species occur in Australian waters. Other countries where several sillaginid species are reported include Philippines, Taiwan and India. Among the Indo-West Pacific sillagos, *Sillago sihama* enjoys the widest distribution.

Sillaginid fishes form small but commercially important fisheries throughout the range of their distribution. They are captured by a variety of gears, such as seine nets, cast nets, gill nets, lift nets, hooks and lines, and trawl net. In the Indo-West Pacific area, the total landings of sillaginids were 16,193 t in 1985 (FAO, 1985). In Australian waters, *Sillago bassensis*, *S. maculata*, *S. ciliata*, *S. schomburgkii*, *S. robusta*, *S. sihama*, *S. analis* and *Sillaginodes punctata* form commercial fisheries. During 1911-1942 period, total whiting landings per year amounted to about 1427 t in Australia (Cleland, 1947). A major share of the catch was contributed by *S. punctata*. Maclean (1973), Penn (1977), Matilda and Hill (1981), Stephenson *et al.* (1982a,b) and

Pollock and Williams (1983) have studied different aspects of the commercial fishery of sand whitings in Australian coasts.

The fact that the taxonomical interest of sand whitings dates back to the early part of the 19th century notwithstanding, the identity and the systematic status of the different species have been a subject of constant dispute. Some of the earlier works on the systematics of sillaginid fishes include that of Cuvier (1817, 1829), Richardson (1842), Gunther (1960), Gill (1862a) and Boulenger (1901). There has been a general consensus that the family Sillaginidae is closest to Sciaenidae. Cleland (1947) had made few considerations about the taxomomy of the sand whitings from New South Wales and Queenland waters. Recently, Mckay (1985) has revised the systematics of Sillaginidae from Indian and Western Pacific oceans, based on the morphology of swimbladder, vertebral counts and cranial osteology. He has described three genera, three subgenera, twenty five species and five subspecies. He has commented that some species are misidentified due to the close external similarities. For example, all the records of *Sillago sihama* from Japan seem to refer to *Sillago japonica* (Mckay, 1985). Systematics of whitings from Taiwan waters were investigated by Shao and Chang (1978, 1979) and Shao *et al.* (1986).

Studies on the biology of sillaginid fishes are rather scanty and the available information is mostly on the Australian species. Cleland (1947) determined the age and growth of *Sillago ciliata* based on the annuli on scales. He observed that the maximum fork length reached after a full year of growth was 220 mm. This species attained sexual maturity for the first time

at 260 mm fork length in the second year. Age and growth studies of *S. sihama* (Mio, 1965) and *S. japonica* (Yu and Tung, 1983) were carried out, respectively, in Japan and Taiwan.

Sand whitings are carnivores which feed on a wide range of benthic and epibenthic prey (Tosh, 1903; Cleland, 1947; Maclean, 1969; Dredge, 1976; Gunn and Milward, 1985). The prevalence of each prey may differ in the diet of each whiting species, but the range of diet is very similar interspecifically. Examination of the stomach contents in *Sillago ciliata* showed only annelids and crustaceans (Tosh, 1903; Cleland, 1947). Macleand (1971) observed that the juveniles of *S. maculata* preferred small crustaceans, while the food of the adult fish consisted mostly of polychaete worms.

Some information is available on the maturation and spawning of commercially important sillaginid fishes from Australia and Japan. In Australia, resproductive biology of *S. ciliata* (Tosh, 1903; Cleland, 1947; Morton 1982), *S. maculata* (Ogilby, 1893; Maclean, 1969), *S. schomburgkii* (Thomson, 1957d; Lenanton, 1969a), *S. analis* (Thomson, 1957d; Lenanton, 1969b; Weng, 1986), *S. robusta* (Grant, 1965) and *S. lutea* (Mckay, 1985) was studied. All these species were found to be protracted breeders, with spawning season lasting for 5 to 8 months. Cleland (1947) found that in *S. ciliata*, spawning season was from August to January in New South Wales, whereas as it was from April to September in Queensland. He has attributed this difference to physiological raciation of the species in the two regions. Most of the species in Australian waters were found to spawn during September to April period.

It seems likely that spawning of whitings take place either in the mouth of the rivers, or more probably, in the open sea (Cleland, 1947; Mckay, 1985).

The size at first maturity of *Sillago robusta*, *S. lutea*, *S. schomburgkii*, *S. analis* and *S. ciliata* were reported to be, respectively, 130 mm, 100 mm (both standard length), 240 mm, 216 mm (both total length) and 260 mm (Fork length). The range of fecundity of *S. schomburgkii* and *S. analis* were, respectively, 30,000-70,000 and 170,000-217,500 (Thomson, 1957d). Recently, Goodall *et al.* (1987) have reported quantitative histology of the seasonal changes in the gonads of *S. ciliata* from Queensland waters.

In Japan, spawning (Kumai and Nakamura, 1977, 1978; Lee, 1979, 1981; Kashiwagi *et al.*, 1984; Lee and Hirano, 1985) and egg development (Ueno and Fujita, 1954) of *Sillago sihama* (apparently misidentification of *S. japonica*, as suggested by Mckay, 1985) were studied. The spawning season of the species in Japan falls between June and September (Lee and Hirano, 1985).

Majority of the sillaginid fishes are found to prefer sandy substratum, while few may occur is silty or muddy substrata (Mckay, 1985). Stephenson and Dredge (1976) observed abundance of *Sillago analis*, *S. ciliata* and *S. maculata* around the mouths of rivers and creeks, which could be attributed to greater food availability, mostly macrobenthos in these areas (Quinn, 1980). *Sillago bassensis* and *S. robusta* were reported to occur in areas of sandy substrata associated with oceanic water with strong currents (Milford and Church, 1977). Weng (1986) studied the distribution, seasonal occurrence and related factors of *S. bassensis*, *S. robusta*, *S. ciliata*, *S. analis* and *S. maculata* in Morton

bay, Queensland. Other works on the ecological aspects of sillaginid fishes include that of Kakuda (1970) and Dredge (1976).

Some workers have studied the chemical constitution of whitings (Integran *et al.*, 1956; Beck, 1956; Hirao *et al.*, 1959; Morris, 1959; Innami and Kubota, Yamakawa *et al.*, 1963; Shimizsu *et al.*, 1969; Sakaguchi and Kawai, 1971; Harada and Yamada, 1973; Eustace, 1974; Ichikawa and Ohno, 1974). Most of these workers deal with inorganic constituents and vitamins. Nogusa (1951,1960) described the chromosomes in *S. sihama*.

Culture of Sillaginids is only is its infant stage. With the increasing reclamation work of coast and water pollution in Japan, the resources of sillaginid fishes have been reported to be declining (Kumai and Nakamura,1977) and artificial propagation has become essential. Experimental culture of *S.sihama* has been initiated in Japan. Fry measuring 50 mm in fork length (average size) were cultured in floating net cage and fed with minced fish meat. In one year time, they grew up to an average size of 168 mm FL and 40.0 g in weight (Kumai and Nakamura, 1978). Lee and Hirano (1981) have suggested a suitable feeding schedule for rearing the larvae of *Sillago* in hatchery. The captive specimen would start spawning as water temperature of the culture medium rises above 20°C (Lee and Hirano, 1985). While studying the salinity tolerance of eggs and larvae of this species in culture conditions, Lee *et al.*(1981) have observed a higher tolerance of fertilized eggs resulting from natural spawning to salinity change than those from induced spawning. They have also observed a better survival of the larvae in lower salinities.

In India, sand whitings occur in near coastal waters, brackishwaters and estuaries along both east and west coasts. They are reported from Hooghly river, Chilka lake, Visakhapatnam, Kakinada, Madras, Mandapam, Cochin, Karwar, Netravathy and Gangolli estuaries and Goa (Sujatha, 1987). They constitute minor, but a fishery of considerable economic importance. Fishing season of *Sillago sihama* at Mandapam extends from May to December (Radhakrishnan, 1957) and at Bombay from July to October (Palekar and Bal, 1955). In Cochin backwaters, *Sillago* is caught only during the south-west monsoon season (Personal observation). Sujatha (1987) reported that fishing season of *Sillago lutea* at Kakinada extends from February to August and that of from March to late June at Kakinada and Visakhapatnam.

According to Krishnamurthy (1957), the *Sillago* landings at Rameswaram island during 1952-53 period was 2.43 t, which was 0.16 percent of total fish catch, and during 1953-54 period, the *Sillago* landings were 16.99 t (1.05 percent of total fish catch). In Pulicat lake, *Sillago sihama* contributes to nearly 2 t per month, forming 2 to 3 percent of the total catch from the lake. This also amounts to about 34 percent of the perch landings of the lake (Kaliamurthy, 1984). *Sillaginopsis panijus* forms part of the catches of bag nets and long lines operated in the rivers and their tributaries in Sunderbans. In 1956, the catches of *S. panijus* from West Bengal and Orissa amounted to 171 t. In the Hooghly Matlah estuary, its annual contribution to the catch from 1963-64 to 1970-71 was 26, 60, 25, 26, 360, 618, 407 and 64 t, respectively. In general, fishery is supported by 0-5 year olds, with a predominance of 1-2 year olds (Gopalakrishnan, 1973; Jhingran and Gopalakrishnan, 1973; Krishnayya, 1963).

Day (1876) was the first worker to study the systematics of Indian sillaginids. He recorded three species from Indian waters. Later, Palekar and Bal (1955) identified one more species. More recent works on the systematics of Indian sillaginid fishes include those of Mckay (1980) and Dutt and Sujatha (1980, 1982). Dutt and Sujatha (1980) are critical of the earlier workers (Radhakrishnan, 1957; Palekar and Bal, 1961; Krishnamurthy, 1969; James *et al.*, 1976; Ramamurthy and Dhulked, 1977) for depending on Day's (1876) work for identification of Sillaginids, despite the increasing evidence that many of the descriptions given by him are inadequate for distinguishing the numerous common and closely related species from many families that are regularly represented in the catches. Nine nominal species belonging to 2 genera and 3 sub-genera are known from Indian waters (Dutt and Sujatha, 1982).

A perusal of the literature on the bioilogy of sillaginids from India shows that most of the works pertain to *Sillago sihama*. Radhakrishnan (1957) determined the age and growth of *S. sihama* based on otolith studies. According to him, the size ranges of the fish during 1st, 2nd, 3rd and 4th years of growth were, respectively, 130-140 mm, 160-200 mm, 200-240 mm and 240-280 mm. He observed that the species attains sexual maturity in the first year at about 130 mm total length. Age and growth of *Sillaginopsis panijus* from Hooghly estuary have been studied (Krishnayya, 1963).

Chacko (1949) observed that *Sillago* seemed to be an omnivorous feeder, browsing among the seaweeds and corals. Radhakrishnan (1957) found

that polychaetes, crustaceans and fishes constituted the principal food materials, besides sea weeds and bivalves in small proportions. Palekar and Bal (1961) studied the food and feeding habits of the Indian sand whiting from Karwar waters. The juvenile fish seems to prefer mostly crustaceans, whereas the adults polychaetes (Krishnamurthy, 1969; James *et al*,1976). *Sillaginopsis panijus* has been observed to feed on crustacea, algae and fish in the Hooghly and Ganges delta (Mookerjee *et al.*, 1946).

Different aspects of reproductive biology, such as spawning season, size at first maturity and fecundity of *S. sihama* from Palk Bay and Gulf of Mannar (Radhakrishnan, 1957), Karwar(Palaekar and Bal, 1961) and Nethravathy and Gangolli estuaries (James *et al*,1976) were studied. Attempts on the induced breeding of this species at Mangalore were unsuccessful (James, 1984). Histological and biochemical investigations of gonadal maturation of sillaginid fishes have not received any attention.

Joshi *et al*,(1953) and Velankar and Govindan (1958) studied the chemical constitution, and Tripathy (1952) the parasitic protozoans of *S. sihama*.

Sillago sihama, though commonly found in marine and estuarine environments, may even ascend rivers (Gunther, 1861; Macleay, 1883). Considering its tolerance to wide ranges of temperature and salinity and also good growth rate and high palatability, *S. sihama* holds bright prospects for aquaculture in the near coastal waters, backwaters, and estuaries. In India, availability of seeds of the Indian whiting has been reported from both the coasts. In Karwar waters, post larval and early juvenile stages in the size range of 20-140 mm are caught in good numbers in December and January (Palekar and Bal, 1960).

Gangolli estuary has been reported to be a good source of *Sillago* seed (Ramamurthy and Dhulkhed, 1977). Post larval stages were abundant off Trivandrum coast in February and April (Gopinath, 1942, 1946). Seeds in the size range of 12-80 mm were collected in good numbers almost throughout the year from the inshore waters of Palk Bay at Pullamadam (James *et al.*, 1984a). In Chilka lake, juveniles of this species in the size range of 15-30 mm are available from October to June (Jones and Sujansinghani, 1954; Kowtal, 1976). These investigations are apparently indicative of the abundance of *Sillago* seeds from the wild.

Sillago sihama has been cultured in salt water ponds, cages and net pens at Mandapam (James, 1984). Average monthly increment in growth in these culture systems were, respectively, 11.4 mm (1.9 g), 10 mm (1.6 g) and 16.8 mm (8.1 g). At Mulky near Mangalore, while culturing this species, an average growth increment of 87 mm in 4 months was noticed (James, 1984). Culture potential of *Sillago vincenti* in the brackishwater bodies of Kerala state has been indicated (Mckay, 1980).

In the area selected for the present work, namely Mandapam and surrounding places in Ramanathapuram district, *S. sihama* forms a minor fishery of some economic importance. As mentioned earlier, in Palk Bay, good seed resources of *Sillago* are available almost throughout the year. This makes the Indian whiting an ideal candidate for culture purpose in the region. Preliminary experiments have shown that they thrive well in net cages (James, 1984). Stocking the seed at the right size and proper management of the culture system may yield good results, and make the culture of *S. sihama* economically viable.

The foregoing brief review of the literature on the investigations carried out on Sillaginids in different regions of Indian and Western Pacific oceans indicates that systematic position of the members of the family Sillaginidae would need further investigations. Proper indentification of the fishes belonging to the family Sillaginidae has not so far been given adequate attention in the area of present study. Although some information is available on their reproductive biology, studies on the development and maturation of gonads at the cellular level as well as biochemical and physiological aspects of gametogenesis have hardly been attempted. Notwithstanding the fact that good quantity of seed is available from the wild at present, there would be greater demand for seed as the culture operation would assume larger proportions in future. This would necessiate production of *Sillago* seed through controlled breeding programme, the success of which would very much depend on the knowledge of its gonadal maturation and gametogenesis. In view of the great culture prospects of *Sillago sihama* in Indian waters, the present study on its systematics, maturation and spawning, histology and histochemistry of gametogenesis and biochemical changes in various tissues during sexual maturation has been taken up. The results of some preliminary experiments carried out on induced maturation and spawning of *S. sihama* are also reported.

PLATE I.

A specimen of *Sillago sihama* (Forskal, 1775)

PLATE I

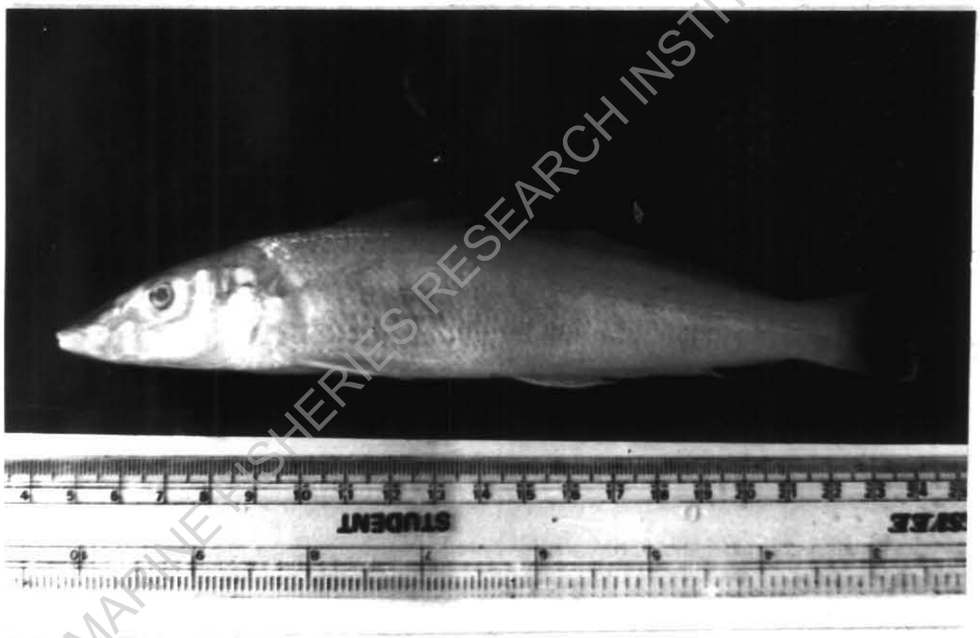
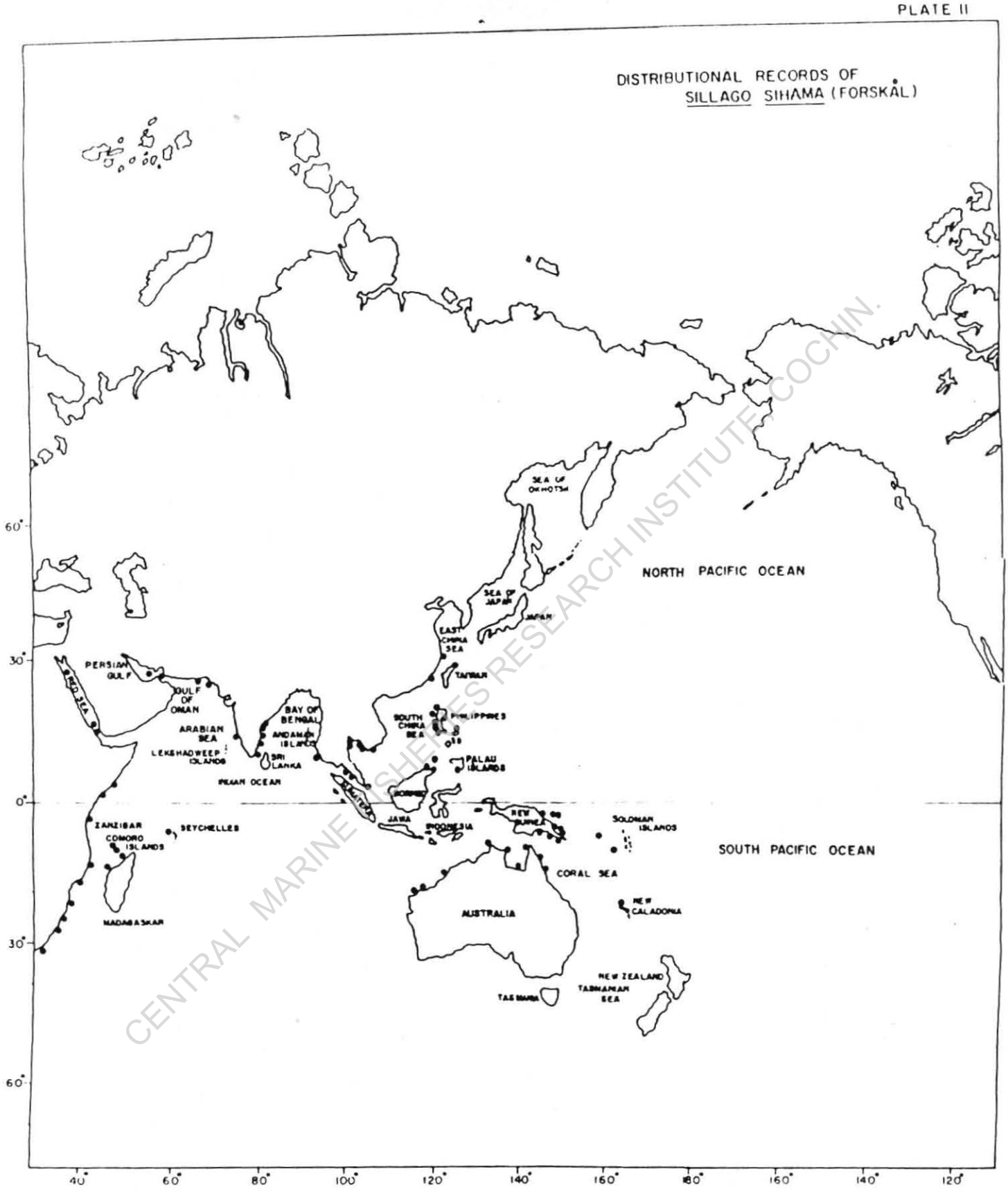


PLATE II

Distributional records of *Sillago sihama* (Forskal).

PLATE II



CHAPTER - II

MATERIALS AND METHODS

2.1. Field Collection

Samples of *Sillago sihama* were collected from selected landing centres in Ramanathapuram district of Tamilnadu (Plate III) during the period April 1984 to March 1986. These collection centres are located along the coast of Gulf of Mannar and Palk Bay. The bay and the gulf are connected by the narrow Pamban pass (Lat. 9° 17'N; Long. 79° 12'E).

During south-west monsoon, the Gulf of Mannar becomes turbulent owing to strong winds and this condition prevails from May to August. In this period waters of Palk Bay are calm. With the onset of north-east monsoon, the opposite conditions exist, with a calm sea in the gulf and turbulent sea in the bay. Fishing operations are largely influenced by these conditions. During south-west monsoon (May to August) fishing operations are concentrated in the Palk bay and during north-east monsoon (September to April) in the Gulf of Mannar.

For the present investigation, samples were drawn from 7 landing centres, 4 of which are situated in the Gulf of Mannar and 3 in the Palk Bay. *Sillago* is caught in gears such as shore seines ('Karavalai' and 'Sippivalai'), shrimp trawl and a kind of stake net ('Kalamkattivalai') in these places. Table-1 provides the information of these landing centres and fishing gears from which samples were collected for the present study.

PLATE III.

Map showing the fish landing centres from which samples of *S. sihama* were drawn during the present study.

PLATE III

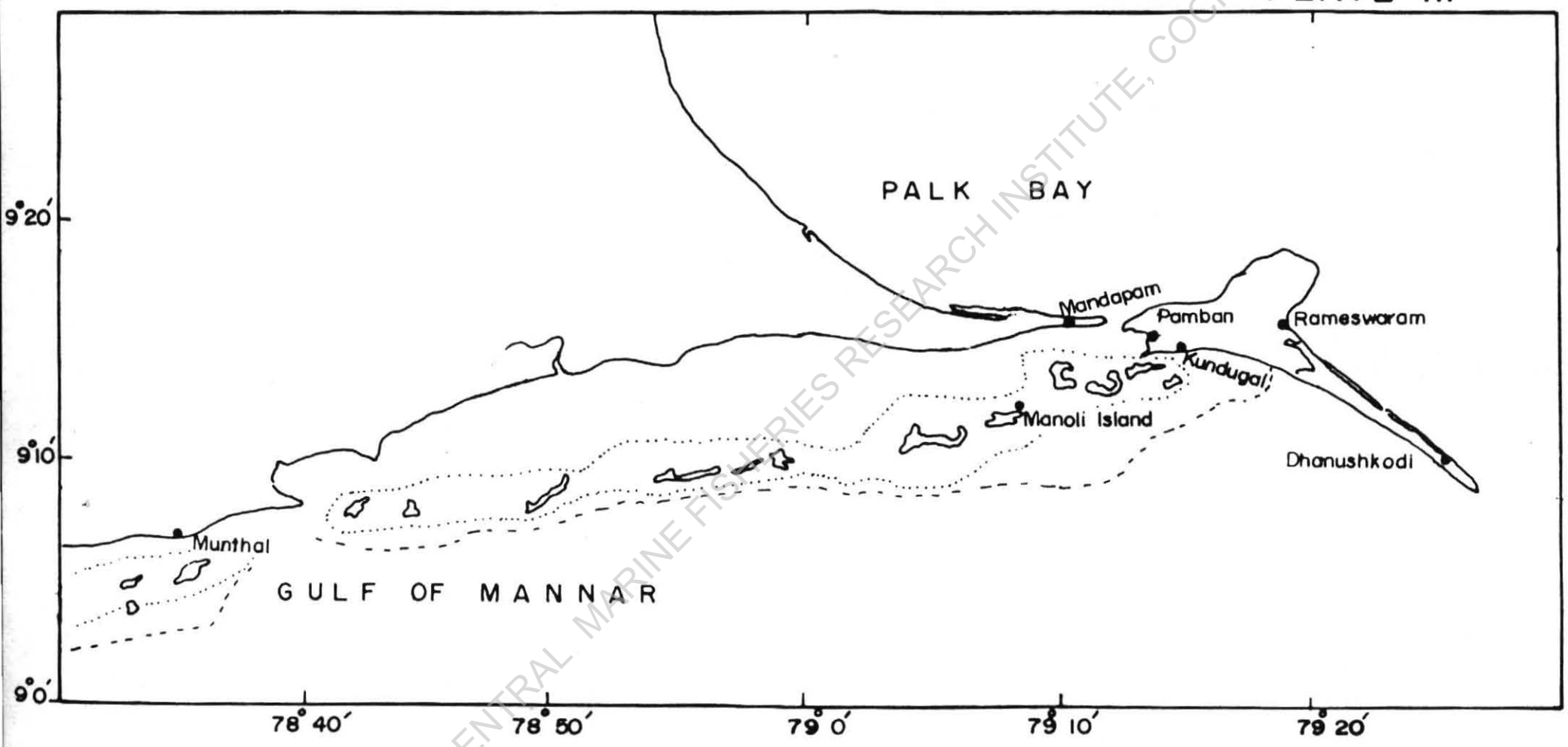


TABLE-I Landing centres and fishing gears from which sampling was done for the present study

Landing centre	Fishing gear	Mean depth of operation
1. Gulf of Mannar		
Kundugal	Shore seine	6 m
Manoli island	Stake net	1 m
Pamban	Shrimp trawl	28 m
Mundal	Shore seine	8 m
2. Palk Bay	Shore seine	6 m
Dhanushkodi	Shore seine	6 m
Rameswaram	Shrimp trawl	15 m
Mandapam	Shrimp trawl	15 m

'Sippivalai' was found to be the most efficient gear to collect *Sillago*. Generally the net is operated by two persons by the side of the 'Karavalai'. While the latter is about to be hauled to the shore, the fishes escaping from it are caught in 'Sippivalai'. *Sillago* buries itself in mud when they feel the 'Karavalai' over it and leave the mud and swim off after the net passes the area. During this time, the fish is trapped in the 'Sippivalai'.

At the collection sites, the blood samples were taken from the live specimens landed by the shore seines. A blood sampling kit was taken to the landing centre for this purpose. The kit contained hypodermic needles (19 and 21 gauges), syringes (GLASS VAN - 1 and 5 ml), sodium heparinate solution, screw-capped vials and distilled water. An ice box was taken which contained crushed ice.

Initially 3 methods of blood collection were tried, with a view to finding out the best method to draw maximum quantity, as it was found that the blood volume of *Sillago sihama* was relatively low. Of the three methods, collection of blood by cardiac puncture and by severing the tail were less effective, while from *artelia caudalis* blood could be collected by introducing the needle through a point just behind the anal opening on the ventral side of the body till it struck against the vertebral column. 1 to 2 ml of blood was obtained from each individual depending on the size.

Blood was drawn using clean glass syringes pretreated with 1% sodium heparinate solution (anticoagulant). The samples were immediately transferred to numbered screw-capped glass vials, which had been previously rinsed with heparinate solution. The vials were placed on crushed ice and taken to the laboratory. The fish were kept in ice contained in the ice box and brought to the laboratory for detailed studies.

2.2. Taxonomy

Taxonomic study was based on the analyses of selected morphometric and meristic characters. Both fresh and preserved (in 5% formalin) specimens were used for the study. Care was taken to select only intact specimens for taxonomic study. The identified specimens belonging to different species have been deposited in the Reference collection museum of the Regional centre of Central Marine Fisheries Research Institute, Mandapam camp.

Meristic and vertebral counting and morphometric measurements were done following the methods of Mckay (1985).

Counts:

The dorsal and anal fin spines and rays were counted. The last pterygiophore of dorsal and anal fins normally supports two rays and were counted as a single element. The anal spines were invariably two in number; the first spine of the anal fin may be reduced in size and required careful dissection in some small specimens.

Lateral line scales bearing pores were counted from the upper margin of the operculum to the caudal flexure at the posterior margin of the hypural.

Transverse scale rows were counted from the origin of the dorsal fin in an oblique row to, but not including, the lateral line scales, and then from the origin of the anal fin obliquely forwards and upwards to the lateral line scales.

Number of cheek scales were counted from below the eye to the margin of the preopercle.

The vertebrae were counted from boiled and defleshed fresh specimens. The axial skeleton was subdivided into three sections, the abdominal vertebrae from the base of the skull to the first heamal arch, the modified vertebrae overlying the swimbladder posteriorly, and the caudal vertebrae bearing straight haemal spines. The conical terminal segment (urostylar vertebrae) was included.

Measurements:

Measurements were made along the longitudinal axis of the body using a fish measuring board. Vernier calipers were used to determine head,

eye, snout and depth measurements. The following body dimensions were chosen to describe the species:

1. Total length (TL): from the tip of snout behind the upper lip to the tip of the upper half of the caudal fin.

2. Standard length (SL): from the tip of snout behind the upper lip to the caudal flexure at the hypural region.

3. Head: from the tip of the snout to posterior margin of the fleshy opercle.

4. Snout: from tip of the snout to anterior fleshy margin of eye.

5. Eye: the horizontal diameter between the fleshy margins of the orbit.

6. Interorbital width: the least width of the bony interorbital space.

7. Snout to ventral fin origin: from the tip of snout to a line perpendicular to the origin of the ventral fin.

8. Snout to first dorsal origin: from tip of snout to a line perpendicular to the origin of the spinous dorsal fin.

9. Snout to second dorsal origin: from tip of snout to a line perpendicular to the origin of the spine preceding the rayed dorsal fin.

10. Snout to anal fin: from tip of snout to a line perpendicular to the origin of the first anal spine.

11. Greatest body depth: depth at middle of body.

12. Least depth of the caudal peduncle.

Morphometric measurements were expressed in percentage of standard length and head length and their range, arithmatic mean, standard deviation, standard error and coefficient of variation were calculated. Percentage of coefficient of variation, if more than 10, indicated that the particular morphometric character was significantly variable.

Swim bladder:

Specimens were dissected by a cut down the middle of the ventral surface from the isthmus to a few millimeters from the vent, thence circumventing anus and urogenital sperture along the side of the vertebral column, to expose the full length of the swimbladder. The gills and viscera were removed and the thin peritoneum carefully pulled away from the surface of the intact swimbladder. Care was taken not to damage any anterior or lateral appendages of the swimbladder, nor break the duct-like process from the ventral surface of the organ to the urogenital aperture.

2.3. Maturation and Spawning

The fish brought from the landing centres, after blotting out water adhering to them, were weighed nearest to 0.1g. Total length and Standard length (in mm) were taken as described under section 2.2. Each fish was then dissected out and the gonads were examined. After recording the colour, shape and size, the gonads were taken out from the body cavity, moisture removed using a blotting paper and weighed nearest to 1 mg.

Reproductive stage of the male fish was determined based on the size, colour and extent in the body cavity of the testes. Reproductive stage of the female fish was determined by ova diameter measurement. Ova from fresh ovaries were only measured in the present study in order to avoid their shrinkage owing to preservation in formalin. A small piece of ovary was taken and teased on a microslide. The ova were carefully separated with fine needles and as evenly as possible spread out on the slide. Diameter measurement of atleast 500 ova from each ovary was carried out under the microscope with an ocular micrometer at a magnification which gave a value

of 16.67 µm to each micrometer division. The ova were taken from the middle portion of the ovary throughout the study, inorder to maintain uniformity in the results.

Spawning season was determined by examining the gonadal maturity of 1184 fishes during April 1984 to March 1985 and 1240 fishes during April 1985 to March 1986.

Gonadosomatic index (GSI), which is the index of maturity condition of the fish, was determined by the following formula.

$$GSI = \frac{\text{Weight of the gonads(g)}}{\text{Body weight of fish (g)}} \times 100$$

Monthly mean GSI values of both male and female fishes were determined.

Size at first maturity, which is defined as the total length (mm) at which 50% of the samples were in mature group, was determined using the samples collected during July 1984 to February 1985 and July 1985 to February 1986, which were found to be the spawning periods in the two successive years. More details about the determination of size at first maturity are given in the relevant chapter.

Ovaries from 17 specimens were taken for fecundity estimation. Ovaries were removed and the excess of moisture was blotted out with a blotting paper. Weight of the ovary was taken nearest to 1 mg. A piece of ovary was taken from the middle portion and weighed on a thin aluminium foil. Weight of the aluminium foil was taken separately. The difference between the two gave the weight of the sample. This sample was preserved in modified Gilson's fluid (100 ml 60% alcohol + 800 ml water + 15 ml 80% nitric acid + 18 ml glacial

acetic acid + 20 g mercuric chloride) in screw-capped glass vials. These vials were periodically shaken to liberate the ova from the ovarian tissue. Microscopic examination has revealed that there is considerable amount of yolk accumulation in the ova measuring more than 0.23 mm in diameter. The ova greater than 0.52 mm in diameter were the transparent ripe ones, some of which could be shed as soon as they are formed. Therefore to avoid underestimation of fecundity, the fully ripe ova were not counted. All the ova measuring between 0.23 and 0.52 mm in diameter were counted for fecundity estimations. The total number of ova in this size group in the whole ovary was determined by the following formula:

$$\text{Fecundity} = \frac{\text{Weight of the ovary (g)} \times \text{Number of ova in the sample}}{\text{Weight of the sample (g)}}$$

A total of 2424 fishes collected from commercial catches landed by shrimp trawlers and shore seines were examined for determining sex-ratio. It was not possible to detect any external characters, such as body proportions, meristic characters or color, which could be useful for sex determination. As fishes were brought to the laboratory for studies, along with other measurements their sex was also noted. The data was tabulated for each month and tested by chi-square (χ^2) test (Snedecor and Cochran, 1968) for statistical significance (to find out whether the ratios were significant at 5% ot 1% levels). To know whether sexes of different length groups deviated from 1:1 ratio, the fishes were classified into 10 mm length groups and tested by chi-square test.

2.4. Histology of the gonads

For histological studies, middle portions of ovary and testis were dissected out from freshly killed specimens and fixed in neutral buffered formalin (NBF), Bouin's fixative or Smith's fluid (Coolidge and Howard, 1979). All the maturity stages of testis and first two maturity stages of ovary were fixed in NBF and Bouin's fixative, while the advanced maturity stages of ovary were fixed in Smith's fluid, since this fixative was found ideal for yolked ova.

After 24 hours of fixation, the tissues were washed under running tap water and stored in 70% ethyl alcohol until further processing. The stored tissues were later dehydrated following the standard procedure in graded alcohol series. The tissues were then cleared in methyl benzoate, impregnated with and embedded in paraffin wax (56-58 degree C melting point). The material was then sectioned at 6-8 µm in a rotary microtome. Initially while sectioning yolky vitellogenic oocytes in the ovary, satisfactory results could not be obtained, presumably due to poor infiltration of wax. The oocytes exhibited extreme wrinkling and collapse of the zona radiata. This problem was overcome to a considerable extent by providing additional support to the yolk by painting the cut surface of the block with a supporting agent (Davis,1977). 2% celloidin in 50% ethnol-ether mixture was painted on the block surface prior to cutting each section.

The sections were spread over slides on which a thin layer of Mayer's glycerol albumen adhesive was applied earlier. This adhesive is a combination of egg white and glycerol in 1:1 ratio. The sections were deparaffinised, hydrated and stained with Ehrlich's haematoxylin and 1% aqueous eosin as counter

stain or Mallory's triple stain. DPX was used as the mounting medium for all the slides.

Measurements were taken using an ocular micrometer whose one division was equivalent to 16.67 µm. Photomicrographs were taken at different magnifications, such as X80, X160, X320 and X640.

2.5. Histochemistry of oocytes

The distribution of specific types of proteins, carbohydrates, lipids and nucleic acids in the oocytes at various stages of maturity were studied using standard histochemical techniques (McManus and Mowry,1960; Pearse, 1968; Coolidge and Howard, 1979).

Tissues were fixed in different fixatives for detecting various constituents. For protein and nucleic acids tissues were fixed in NBF, for carbohydrates in Telly's and for lipids in Formol-calcium. For proteins, carbohydrates and nucleic acids, tissues were processed embedded and sectioned in the manner similar to that used for histology.

Preparation of tissue sections for demonstrating lipids was done in the following way:

- Formol-calcium fixed tissues were washed in tap water.
- Infiltrated overnight in 12.5% gelatin and changed and kept in 25% gelatin overnight.
- cryocut sections were prepared using a histostat. Tissue was placed on the block holder and a drop of distilled water was placed and allowed to freeze at -20 degree C.
- 10 - 12 µm thick sections were cut; an antiroll guide was used to prevent curling of the individual sections.

- Sections were transferred to the slide by using a fine camel-hair brush

- An adhesive fixative was applied on to the sections and air dried. The fixative consists of 80 ml of 100% alcohol, 20 ml of 10% formalin and 10 ml of glacial acetic acid.

Blocking procedures were carried out to prove the presence of the specific reactive group and the removal of interfering groups. The histochemical tests and the corresponding blocking procedures are given in the relevant chapter.

2.6. Biochemistry

Specimens for biochemical analyses were collected mostly from shore seines, since they land live fishes. Arrangement was also made with commercial trawler crew to put the fish in ice as soon as they were caught in their boats and bring them to the shore in fresh condition. 40 fishes each from various maturity stages of female and male were collected for studying the biochemical composition of liver, muscle, gonads and blood.

Tissue analyses:

Moisture, total carbohydrates, protein, glycogen, lipid and cholesterol were estimated in the liver, muscle and gonad tissues of the species at different stages of maturity. The muscle tissue was dissected out without skin from just below the origin of first dorsal fin of each fish. Immature gonads of each sex were taken from 4 to 5 individuals and pooled together and weight was taken. The tissues were weighed nearest to 0.1 mg. Estimations were carried out on both fresh and dry tissues.

For estimating the tissues on dry weight basis, they were dried in an oven at 80-90 degree C for 48 hours. Then they were transferred to a desiccator, containing anhydrous $CaCl_2$ and kept there till they attained constant weight. Difference in fresh and dry weights of the tissues gave the amount of moisture. The tissues once attained constant weight, were taken out of the desiccator and powdered with a mortar and pestle and used for analyses.

For total carbohydrate estimation, about 20-25 mg of each tissue was taken. The tissue was dissolved in 5 ml of 10% KOH by heating at 60-80 degree C for about 20 minutes. 2 ml of this solution was taken, to which 0.1 ml phenol (98%) and 5 ml concentrated sulphuric acid were added. The iontensity of colour developed was read at 540 nm (Dubois *et al.*, 1956) using ECIL senior spectrophotometer.

For protein and lipid estimations, about 40-60 mg of each tissue was taken. The tissue was homogenised with a mixture of chloroform methanol in the ratio of 2:1 (V/V) and centrifuged. The precipitate was used for protein estimation and the supernatant for lipid.

The precipitate was dissolved in 5 ml IN NaOH. Protein was estimated in 1 ml of this solution by the Folin-Ciocalteu Phenol method (Lowry *et al.*, 1951). Bovine serum albumin was used as the standard. The intensity of the colour developed was read at 700 nm.

To the supernatant, 0.2 volume of distilled water was added and mixed thoroughly. This solution was separated into methanol phase and chloroform phase in a separating funnel. The chloroform phase was collected and the solvent allowed to evaporate. The residual lipid was estimated gravimetrically (Folch *et al.*, 1957).

For glycogen estimation about 90-100 mg of each tissue was taken. After deproteination with 5% TCA and removal of lipids by chloroform-methanol extraction, the resultant precipitate was dissolved in 1 ml distilled water. To this was added 9 volumes of anthrone reagent and kept in boiling water bath for about 15-20 minutes. Then it was cooled and the intensity of the colour developed was read at 620 nm (Caroll *et al.*, 1956).

To estimate the cholesterol, about 40-60 mg of tissue was homogenised with 5 ml of glacial acetic acid and centrifuged. To 1 ml of the supernatant, 4 ml of ferric chloride reagent was added and the mixture kept in ice. To the cooled mixture 4 ml of concentrated sulphuric acid was added and the colour developed was read at 540 nm (Varley, 1962).

Plasma analyses:

The samples of blood collected from the specimens at the collection sites were transported to the laboratory in an ice box containing ice. In the laboratory, the blood samples were centrifuged at 3000 rpm for 10 minutes and the supernatant plasma was separated. Since the blood obtained from individual fish was less, plasma from 3-4 fishes belonging to the same maturity stage and collected from the same landing centre during the same month, were pooled together. Each sample was triplicated for all the tests.

Glucose content in blood plasma was determined by Nelson (1944) and Somogyi (1945) method. 0.1 ml of plasma was mixed with 1.5 ml of distilled water and to this 0.2 ml of Barium hydroxide solution was added. After thorough mixing, 0.2 ml of Zinc sulphate solution was added. The mixture was centrifuged at 3000 rpm. To 1 ml of the supernatant, 1 ml of

Tartrate reagent was added and mixed. This mixture was heated for 20 minutes in boiling water bath and later cooled. 1 ml Arsenomolybdate colour reagent was added and the colour developed was read at 530 nm.

For protein estimation, 0.2 ml of the plasma was treated with 1.8 ml of 80% ethanol and centrifuged at 3000 rpm for 5 minutes. The precipitate was dissolved in 5 ml of IN NaOH and 1 ml of this solution was taken for estimating protein by the Folin-Ciocalteu phenol method (Lowry *et al.*,1951). After 20 minutes, the intensity of the colour developed was read at 700 nm. Bovine serum albumin was used at the standard.

Lipids were estimated as per the method given by Folch *et al.*,(1957) 0.4 ml. of the plasma was extracted with chloroform-methanol mixture (2:1 V/V) and the extract thus obtained was mixed with a few drops of 0.9% NaCl and allowed to Separate into two layers in a separating funnel. The lower phase containing chloroform and lipids was collected and the lipid was estimated gravimetrically after evaporating the chloroform at 30 degree G in a vacuum dessicator.

Cholesterol was estimated by the ferric chloride acetic acid method (Varley, 1962). 0.1 ml of plasma was treated with 10 ml of ferric chloride-acetic acid reagent for 3 to 4 hours and centrifuged at 3000 rpm for 5 minutes. To 5 ml of the supernatant, 3 ml of concentrated sulphuric acid was added and the colour intensity developed was read at 560 nm. Chloroform (extra pure grade) was used as the standard.

The materials and methods for preliminary studies on the induced maturation and spawning' are given in the relevant chapter.

CHAPTER - III

TAXONOMIC CONSIDERATIONS OF THE SILLAGINID FISHES FROM PALK BAY AND GULF OF MANNAR

There has been divergence of opinion regarding the actual systematic position of the family Sillaginidae. Initially, Cuvier (1817) included it under Gobioides, but later transferred to the Percoides. While the sillaginids were placed under Uranoscopidae by Richardson (1842), Gunther (1860) and Day (1876) felt that *Sillago* should belong to the family Trachinidae due to the number of vertebrae and long anal fin. Gill (1862a) found that sillaginids resemble some percoides, particularly *Acerina schraitzer*, though there are some morphological and anatomical characters by which they could be easily distinguished. Boulenger (1901) placed the family Sillaginidae close to the Sciaenidae. The genus *Aspro*, in particular, seemed to resemble *Sillago* in several respects. Mckay (1985) has stated that *Aspro* is the closest relative to the sillaginids. He has revised the systematic position of the fishes belonging to the family Sillagindae from Indian and Western Pacific oceans and has described 3 genera, 3 sub-genera, 25 species and 5 sub-species from these regions. Shao *et al.* (1986) has revised the systematic position of the Taiwanese sand whitings.

From Indian waters, Day (1876) recorded 3 species of the genus *Sillago*, namely *S. domina*, *S. sihama* and *S. maculata*. It has since been established that *S. domina* is a synonym of *Sillaginopsis panijus* (Hamilton and Buchanan, 1822). Later Palekar and Bal (1955) identified one more species, *S. chondropus* from Bombay waters. Mckay (1980) recorded 2 genera and 5 species of sand whitings from Indian waters, and opined

that there were actually more species than have so far been described. Dutt and Sujatha (1980) recorded 7 species from Visakhapatnam waters and in 1983 added one more species to the list.

Taxonomic study was included in the present work considering the prevailing confusion in the correct identification of Sillaginid fishes. Hitherto, no detailed taxonomic study of sillaginids has been carried out in the area of the present investigation. Hence it was felt necessary to confirm the identity of the species without any mistake for the work on reproductive aspects.

OBSERVATIONS

During the present study, I genus *(Sillago)*, 3 sub-genera *(Sillago, Sillaginopodys and Parasillago)* and 6 species *(S. chondropus, S. sihama, S. indica, S. vincenti, S. argentifasciata and S. soringa)* of the family Sillaginidae were collected from different landing centres of Palk Bay and Gulf of Mannar. They were identified based on the descriptions of Mckay (1985) and Sujatha and Dutt (1985). The details of the morphometric measurements and meristic characters of these six species are given in Tables from 2 to 8. *Sillago sihama* was the most dominant species in the present study area, accounting for about 60% of all the sillaginids in the commercial landings.

Genus *Sillago* Cuvier, 1817

Sillago Cuvier, 1817, type by subsequent designation, Gill, 1861, *Sillago sihama* (Forskal, 1775).

Diagnosis:

Sillaginidae in which the swimbladder is present, variously formed, simple or complex, with a mediantubular duct-like process normally present on the ventral surface, lateral line scales 50-84. Dorsal spines 10-13, normally 11 or 12.

Key to the subgenera and species of genus *Sillago* collected during the present study:

1. - Ventral spine very small and situated at the base of a thickened club-shaped outer ventral ray; swimbladder reduced, no median tubular duct-like process, no modified caudal vertebrae..... *Sillaginopodys*

- Ventral spine normal, swimbladder is not reduced; median tubular duct-like process present; modified caudal vertebrae present or absent..... 2

2. - Swimbladder divided posteriorly into two tapering extensions; modified caudal vertebrae present .....*Sillago*..... 3

- Swimbladder with posterior extension single and tapering to a fine point, or round; modified caudal vertebrae present or absent.....
..... *Parasillago*..... 4

3. - Vertebrae 33. Dorsal spine XI. 2 series of scales on the cheek.....
..... *Sillago sihama*

- Vertebrae 34. Dorsal spine XI. 3 series of scales on the cheek .....*Sillago indica.*

4. - Second dorsal fin with atleast 5 rows of dusky black or black-brown spots that may be quite separate or somewhat confluent..... ..... *Sillago vincenti*

- Second dorsal without any distinct rows of pigment spots ..... 5.

5. - A wide, brilliant, silvery longitudinal band on each side of the body. Median tubular duct-like process of the swimbladder absent..... ..... *Sillago argentifasciata.*

- Silver longitudinal band absent along the sides of the body. Median tubular duct-like process of the swimbladder present ..... 6.

6. - Swimbladder with three anterior extensions, the middle one projecting forwards and the antero-lateral ones recurved backwards for a short distance along the sides ..... *Sillago soringa*

Sillago (Sillaginopodys) chondropus (Bleeker, 1849)
(Plate IV, Fig. 1)

Material examined: 4 specimens: Mundal; 160 mm, female, 19.12. 1985; 155 mm, female, 19.12.1985; 165 mm, female, 12.3.1986; 150 mm, male, 12.3.1986.

Description:

Dorsal fins XI-XII. I. 21; anal fin II, 23. Lateral line scales 70-71; TR 6 above, 10-11 below. Cheek scales 3 rows, all ctenoid.

Proportional dimensions as percent of SL : greatest depth of body 15.2 - 16.1; head length 24.9 - 25.3; snout tip to ventral fin origin 26 - 27.3; snout tip to spinous dorsal fin origin 27.9 - 29; snout tip to second dorsal fin origin 50.3 - 51.3; snout tip to anal fin origin 47.5 - 50; least depth of caudal peduncle 8 - 8.5.

Proportional dimensions as percent of head : length of snout 32.5 - 34.2; horizontal diameter of eye 19.5 - 22.5; least width of interorbital 15 - 17.1.

Vertebrae : 13 abdominal, 22 caudal, total 35.

Colour : Pale sandy brown above, paler below, scale margins dusky; a dull silver-grey mid-lateral band usually present, frequently with a wide dusky band below on lower sides. Fins hyaline, the spinous dorsal tinged brown with a fine dusting of black spots at the tip.

Swimbladder : Commences as a very flattened presumably non-functional structure just behind the axis vertebrae and then rather abruptly narrows to a fine point terminating on the ninth abdominal vertebra. No median tubular duct - like process from the ventral surface is present, as the posterior extension terminates well before the first haemal arch; modified caudal vertebrae are absent.

Distribution:

South Africa, Mozambique, West Pakistan, India, Burma, Indonesia, New Guinea, Thailand and Philippines.

Remarks:

This species can easily be identified by the club-shaped first ventral fin ray. The reduced swimbladder and modified ventral fin indicates that

this species is demersal and may use the ventral fin pads somewhat like sled runners on the bottom (Mckay, 1985).

Sillago (Sillago) sihama (Forskal, 1775)
(Plate IV, Fig. 2)

Material examined:

5 specimens: Mandapam: 170 mm, female, 2.1.1986; 148 mm, female, 2.1.1986; 140 mm, female, 2.1.1986; 155 mm, 13.2.1986; 156 mm, male,13.2.1986.

4 specimens : Rameswaram : 164 mm, female, 18.7.1984; 134 mm, male, 25.7.1984; 112 mm, female, 7.2.1985; 143 mm, male, 29.11.1985;

2 specimens : Pamban : 155 mm, male, 10.9.1985; 132 mm, male, 6.2.1986;

2 specimens : Mundal : 135 mm, female, 19.12.1985; 120 mm, female, 12.3.1986.

1 specimen : Kundugal : 149 mm, male, 30.5.1985;

2 specimens : Dhanushkodi : 110 mm, female, 17.7.1985; 136 mm, female, 17.7.1986.

Description:

Dorsal fins XI, I, 21-22; anal fin II, 23. Lateral line scales 69-70. TR 5-6 above, 10-12 below. Cheek scales 2 rows, all cycloid.

Proportional dimensions as percent of SL : Greatest depth of body 16.4-20; head length 25-29.4; snout tip to ventral fin origin 27.4-32.3; snout tip to spinous dorsal origin 32.8-34.9; snout tip to second dorsal origin 53.7-57.3; snout tip to anal fin origin 54.7-58.3; least depth of caudal peduncle 7.1-8.3.

Proportional dimensions as percent of head: length of snout 36.8-42.9; horizontal diameter of eye 21.9-25.6; least width of interorbital 16.3-21.4.

Vertebrae : 14 abdominal, 4-5 modified, 14-16 caudal Total - 34.

Colour : Body light tan, silvery yellow-brown, sandy-brown, or honey coloured; paler brown to silvery white below; a mid-lateral, silvery, longitudinal band normally present; dorsal fins dusky terminally with or without rows of dark brown spots on the second dorsal fin membrane; caudal fin dusky terminally; no dark blotch at the base of the pectoral fin; other fins hyaline, the anal fin frequently with a whitish margin.

Swimbladder : Two anterior extensions extend forward and diverge to terminate on each side of the basioccipital above the auditory capsule; two lateral extensions commence anteriorly, each sending a blind tubule anterolaterally and then extending along the abdominal wall below the investing peritoneum to just posterior of the median tubular duct - like process; two posterior tapering extensions of the swimbladder project into the caudal region, one usually longer than the other. The lateral extensions are normally convoluted and have blind tubules arising along their length but in smaller examples may be more or less convoluted with fewer or no blind tubules. Though some variations were found in the shape of the lateral extensions, all specimens examined have the lateral extensions convoluted to some extent.

Distribution:

A wide ranging species throughout the Indo west-Pacific region (Plate II)

Remarks:

S. sihama is commonly confused with a number of uniform-coloured whiting species. The identification is based mainly on swimbladder, in addition

to lateral line scale and fin ray counts.

Sillago (Parasillago) indica Mckay, Dutt and Sujatha, 1985
(Plate IV, Fig. 3)

Material examined :

4 specimens : Rameswaram : 150 mm, male, 7.2.1985; 152 mm, female, 25.5.1985; 141 mm, female, 25.5.1985, 152 mm, female, 1.2.1986;

3 specimens : Pamban : 125 mm, male, 10.9.1985; 144 mm, female, 10.9.1985, 193 mm, female, 6.2.1986.

2 specimens : Mandapam : 165 mm, female, 2.1.1986; 144mm, male, 2.1.1986.

Description:

Dorsal fins XI, I, 21-22; anal fin II 21-23; lateral line scales 70-76. TR 6 above, 11-12 below. Cheek scales in 3 rows, all cycloid except for occasional ctenoid scale posteriorly.

Proportional dimensions as percentage of SL: Greatest depth of body 18.4-21.2; head length 28-29.1; snout tip to ventral fin origin 29.9-31.2; snout tip to spinous dorsal origin 33.3-34.4; snout tip to second dorsal origin 52.8-57; snout tip to anal fin origin 53.9-56.8; least depth of caudal peduncle 7.1-8.3.

Proportional dimensions as percentage of head length : Length of snout 36.4-39.5; horizontal diameter of eye 18.6-22; least width of interorbital 18.2-20.9.

PLATE IV

Sillaginid fish species with their swimbladders

Fig.1. *Sillago (Sillaginopodys) chondropus* (Bleeker, 1849)

Fig.2. *Sillago (Sillago) sihama* (Forskal, 1775)

Fig.3. *Sillago (Parasillago) indica* Mckay, Dutt and Sujatha,1985.

PLATE IV

Fig. 1

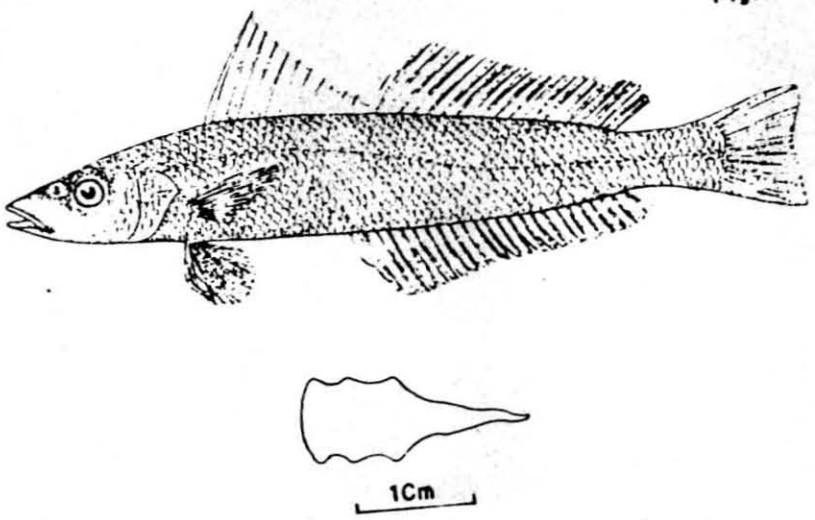


Fig. 2

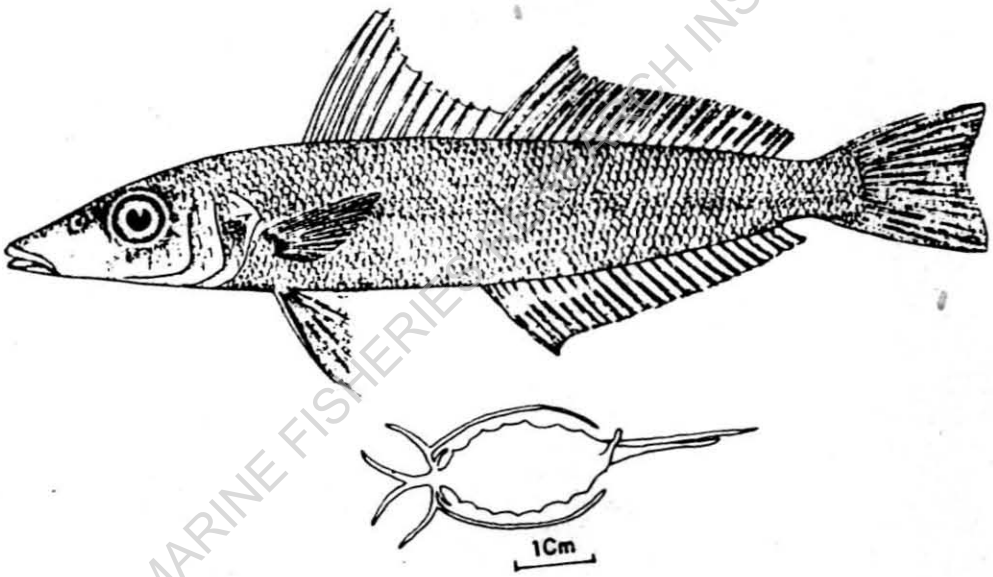
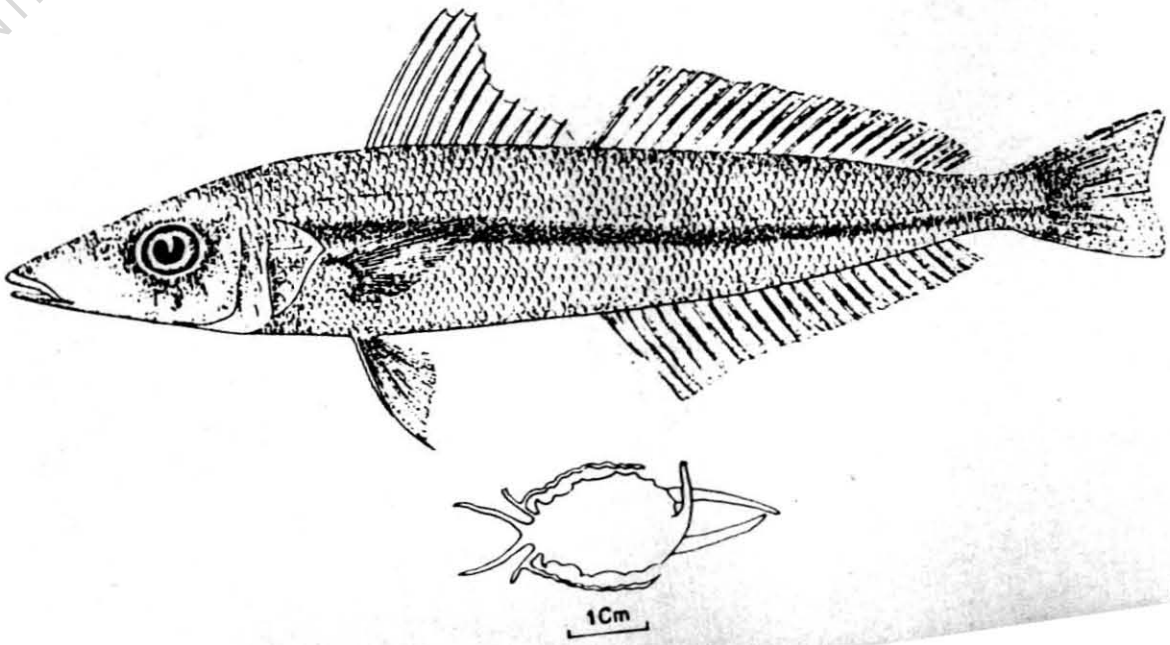


Fig. 3



Vertebrae : 14 abdominal, 3-4 modified, caudal 16-17, total - 34.

Colour : Body light tan with a dark brown to blackish band commencing behind the upper part of the opercle and curving down below the lateral line for approximately two-thirds its length and then continuing slightly below or on the lateral line as a more or less broken band or as distinct elongate spots or blotches, to hypural flexure; head and cheeks with fine black dots; belly and lower sides may be densely dotted, almost blackish; interspinous membranes of first dorsal fin with very numerous black dots; interradial membranes of second dorsal and anal fin dusted with black dots, most concentrated immediately before each ray; caudal dusted with black, lower lobe may be blackish.

Swimbladder: This species has the stoutest swimbladder. It is somewhat similar to that of *S. sihama*. But the third pair of tubular extensions at the anterior end are the longest and distinctly more convoluted than in *S. sihama*. The longer left postcoelomic extension extends to the third or fourth caudal vertebra.

Distribution:

East and west coasts of India.

Remarks:

Sillago indica was earlier reported as *Sillago parvisquamis* by Dutt and Sujatha (1980). The swimbladder of this species is having two distict postcoelomic extensions and thus would belong to the subgenus *Sillago*. But Mckay (1985) has placed this species under the subspecies *Parasillago*, which has only a single postcoelomic extension for the swimbladder. Dr. Dutt

and Dr. Sujatha (Personal communications, 1988) have clarified that Mckay has described the swimbladder (Mckay, 1985, p. 39) of *S. indica* wrongly.

Sillago (Parasillago) vincenti Mckay, 1980
(Plate V, Fig. I)

Material examined:

4 specimens : Pamban : 211 mm, male, 11.12.1984; 216 mm, female, 11.12.1984; 217 mm, female, 13.12.1985; 188 mm, female, 13.12.1985;

1 specimen : Manoli island: 204 mm, female, 3.4.1984.

Description :

Dorsal fins XI, I, 21-22; anal fin II, 22-24; lateral line scales 71-73; TR 5-6 above, 13-14 below; cheek scales in 2 rows all cycloid.

Proportional dimensions as percent of SL: Greatest depth of body 16.7-19.8; head length 26.1.29; snout tip to ventral fin origin 26.5.30; snout tip to ventral fin origin 26.5-30; snout tip to spinous dorsal origin 30.8-35; snout tip to second dorsal origin 53.6-54.8; snout tip to anal fin origin 53.5-55.6; least depth of caudal peduncle 6.2-6.9.

Proportional dimensions as percent of head length : Length of snout 40-46.2; horizontal diameter of eye 16.4-22.2; least width of interorbital 16.4-19.

Vertebrae : 14 abdominal, 4-6 modified, 14-16 caudal, total - 34.

Colour : Body light olive above; belly white; margins of scales darker; spinous dorsal hyaline with the tip of membranes dusky or blotched. Soft dorsal hyaline with 5-7 rows of blackins spots; anal fin hyaline to milky white.

Swimbladder : The anterior extremity has a very short bulbous projection with one to three anterolateral lobate or recurved projections. The posterior postcoelomic extension is single and tapers to a point; a median tubular duck like process is present on the ventral surface and continues to the vent.

Distribution:

Estuarine areas of Kerala and very shallow waters of the Gulf of Mannar.

Remarks:

The species is very similar in external morphology to *S. sihama*. A dissection of the posterior part of the swimbladder is required for field identification. *Sillago vincenti* was collected only from shallow waters is Manoli island and few places near Pamban in the Gulf of Mannar, where a type of stake net (*Kalamkattivallai*) is operated.

Sillago (Parasillago) argentifasciata Martin and Montalban, 1935

(Plate V, Fig. 2)

Material examined:

4 Specimens : Rameswaram : 150 mm, female, 31.12.1985; 147 mm, male, 31.12.1985; 148 mm, female 1.2.1986; 133 mm, female, 10.2.1986;

3 specimens : Pamban : 144 mm, male, 7.12.1985; 160 mm, female, 4.1.1986; 163 mm, male, 6.2.1986.

Description:

Dorsal fins XI, I, 17-18; anal fin II, 16-17; lateral line scales 62-68; TR 5 above, 8/9 below; cheek scales 3 rows all ctenoid.

Proportional dimensions as percent of SL: Greatest depth of body 18.8-20.3; head length 29.3-30.1; snout tip to ventral fin origin 30.6-31.9; snout tip to spinous dorsal fin origin 33.3-35.3; snout tip to second dorsal fin origin 56-57.9; snout tip to anal fin origin 56-58.7; least depth of caudal peduncle 8-8.3.

Proportional dimensions as percent of head length: Length of snout 38.5-42.9; horizontal diameter of eye 27.3-29.5; least width of interorbital 17.9-18.8.

Colour: Dull silvery white; a well-pronounced, brilliant, silvery, longitudinal band, widest between the anterior portions of anal and second dorsal, runs on side from above base of pectoral to base of caudal; anteriorly this band is below the lateral line and posteriorly its upper edge touches it; breast and opercle brilliant silvery; upper portion of each dorsal spine and ray sparsely dotted with blackish; all other fins hyaline.

Swimbladder: The swimbladder is lanceolate and the anterior edge is slightly complex. Anterolaterally, there are four pairs of short extensions. The edge of the swimbladder behind them is smooth. The single postcoelomic extension is relatively long and pointed and terminates at the ninth or tenth

caudal vertebra. The median tubular duct-like process is absent on the ventral surface.

Distribution:

Lumbucan Island, Philippines and east and west coasts of India.

Remarks:

Mckay (1985) feels that *Sillago argentifasciata* may prove to be a junior synonym of *Sillago ingennua*, a new species described by him from Australian waters. The fin ray counts and lateral line scale counts of *S. ingennua* agree to some extent with those of *S. argentifasciata*, but the well defined midlateral silvery band characteristic of the latter is absent in the former. Dr. Sujatha (personal communication, 1988) remarked that Mckay had erroneously synonimised *S. argentifasciata* to *S.ingennua*.

Sillago (Parasillago) soringa Dutt and Sujatha, 1983
(Plate V, Fig. 3)

Material examined:

2 specimens : Rameswaram : 132 mm, female, 31.12.1985; 120 mm, male 13.2.1986;

1 specimen: Mandapam : 115 mm, male 2.1.1986;

2 specimens : Pamban: 135 mm, female, 4.1.1986; 125 mm, female, 7.3.1986.

PLATE V

Sillaginid fish species with their swimbladders

Fig.1. *Sillago (Parasillago) vincenti* Mckay, 1980

Fig.2. *Sillago (Parasillago) argentifasciata* Martin and Montalban, 1935.

Fig.3. *Sillago (Parasillago) soringa* Dutt and Sujatha, 1983.

PLATE V

Fig. 1

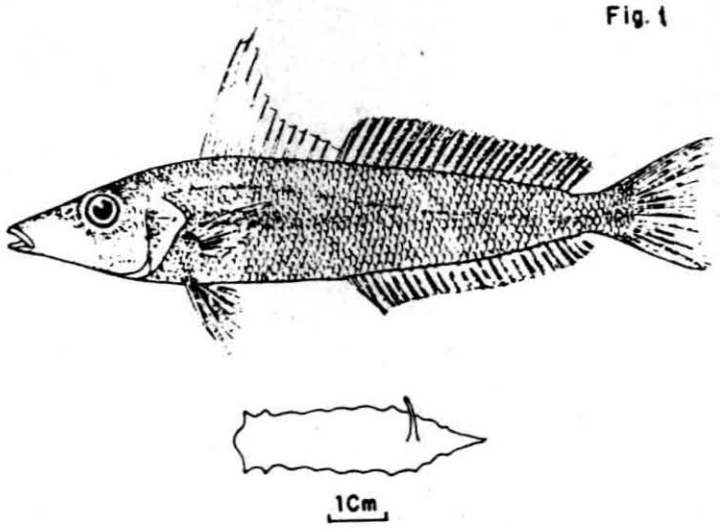


Fig.2

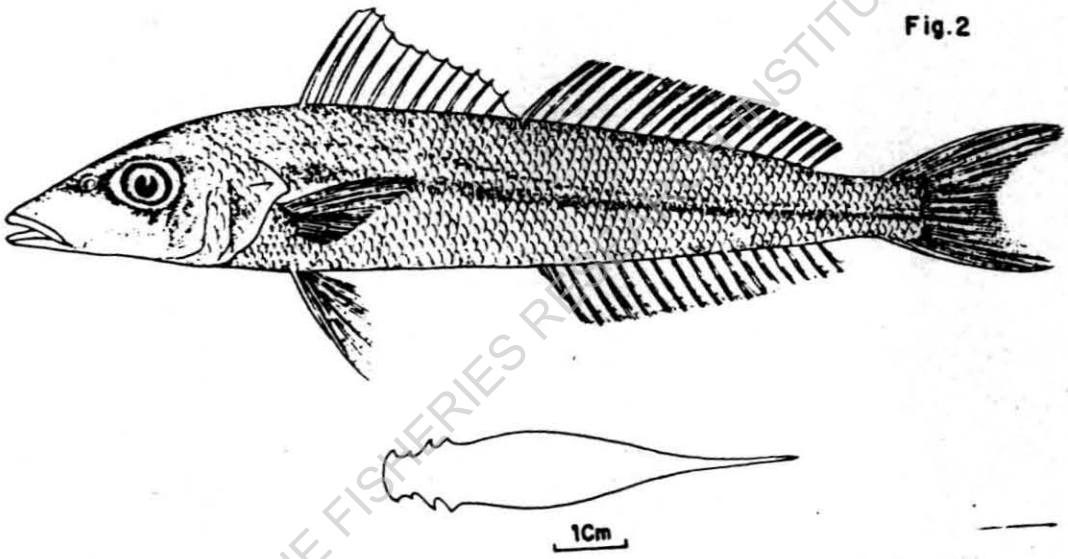
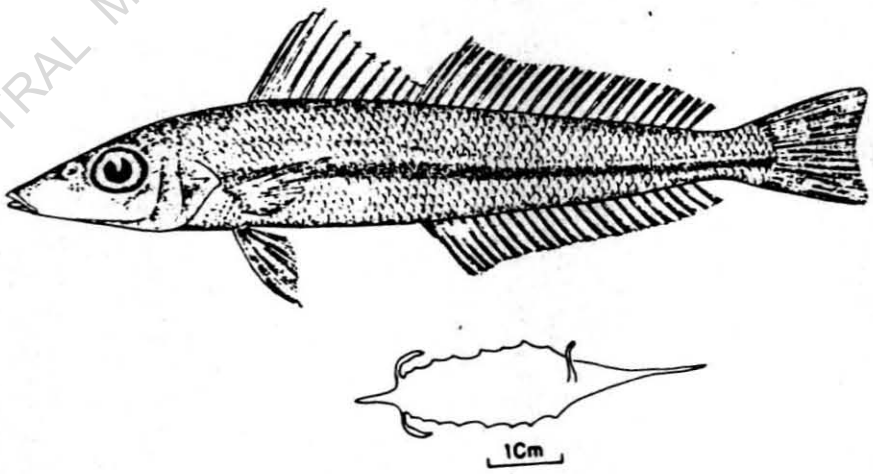


Fig.3



Description:

Dorsal fins XI, I, 21; anal fin II, 22; lateral line scales 64-68; TR 3-4 above, 9-10 below; cheek scales in 2 rows, upper row cycloid, lower ctenoid.

Proportional dimensions as percent of SL: Greatest depth of body 17.4-19.2; head length 27.8-28.8; snout tip to ventral fin origin 30.3-31.3; snout tip to spinous dorsal fin origin 31.2-35; snout tip to second dorsal fin origin 56.1-58.3; snout tip to anal fin origin 54.6-56.3; Least depth of caudal peduncle 6.4-6.96.

Proportional dimensions as percent of head length : Length of snout 37.5-39.5; horizontal diameter of eye 24.3-29.4; least width of interorbital 18.8-22.2.

Vertebrae : 13 abdominal, 5-6 modified, 14-15 caudal, total - 34.

Colour : Dorsal side and upper flanks grey brown, becoming paler laterally; lower flanks and ventral side milky white. Spinous dorsal with minute discrete black dots on membrane; they are more numerous towards the distal half especially in the anterior half of the fin. In the soft dorsal, running parallel to and close to the anterior edge of each ray, is a more or less continuous grey band. The membrane of anal fin is also provided with minute black dots, but to a lesser extent than the spinous dorsal. Pectorals and ventrals hyaline with golden tinge. Caudal hyaline, with fine black dots.

Swimbladder : Lanceolate, with a median finger - like extension and a pair of recurved extensions at anterior end; the swimbladder bears a single tapering postcoelomic extension and a blind tubular duct-like process, which arises

from the middle of its ventral side, about 4/5 the distance from its anterior end, to terminate blindly near the vent.

Distribution:

Visakhapatnam waters, Palk Bay and Gulf of Mannar.

Remarks:

Sillago soringa resembles *S. sihama* and *S. vincenti* apparently been confused with them. But based on swimbladder, they could be distinguished from one another. Mckay (1985) presumes that *S. asciatica*, a new species described by him from Thailand waters, could be synonimous with *S. soringa*. However, the pair of recurved extensions of swimbladder is longer in *S. asciatica* extending of half of the length of swimbladder.

TABLE 2. Morphometric characters of *Sillago chondropus* (Bleeker, 1849)
(all measurements in mm)

Characters	1	2	3	4	Range %	Mean %	Standard Deviation	Standard Error	Coefficient of variation
Standard length	160	155	165	150	In Standard length				
Greatest depth of body	25	25	25	24	15.15 - 16.13	15.72	0.44	0.22	2.80
Head length	40	39	41	38	24.85 - 25.33	25.09	0.21	0.11	0.84
Snout tip to Ventral fin Origin	42	41	45	39	26.00 - 27.27	26.49	0.55	0.28	2.08
Snout tip to spinous dorsal Origin	46	45	46	43	27.88 - 29.03	28.58	0.49	0.24	1.73
Snout tip to second dorsal origin	81	79	83	77	50.30 - 57.33	50.81	0.44	0.22	0.87
Snout tip to anal fin origin	76	75	82	75	47.50 - 50.00	48.90	1.16	0.58	2.38
Least depth of caudal peduncle	13	13	14	12	8.00 - 8.48	8.25	0.22	0.11	2.70
					In head length				
Length of Snout	13	13	14	13	32.50 - 34.21	33.55	0.81	0.41	2.40
Horizontal diameter of eye	9	8	8	8	19.51 - 22.50	20.89	1.25	0.63	5.97
Least width of interorbital	6	6	7	6	15.00 - 17.07	15.81	0.90	0.45	5.69
Sex	F	F	F	M					
Locality	MDL	MDL	MDL	MDL					

MDL=Mundal

TABLE 3. Morphometric characters of *Sillago sihama* (Forskal, 1775)

(all measurements in mm)

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Range %	Mean %	Standard Deviation	Standard Error	Coefficient of variation
Standard length	170	148	164	155	135	149	132	134	112	143	140	155	156	110	136	170	Standard length				
Greatest depth of body	28	26	32	26	27	25	26	22	19	25	27	26	31	19	26	24	16.42 - 20.00	18.12	1.43	0.36	7.90
Head length	43	37	46	40	39	38	38	36	32	37	39	45	44	28	40	31	25.00 - 29.41	27.15	1.59	0.40	5.84
Snout tip to ventral fin origin	48	44	50	46	37	45	41	39	32	45	45	50	49	33	39	38	27.41 - 32.26	30.13	1.47	0.37	4.87
Snout tip to spinous dorsal origin	56	50	56	52	45	51	46	44	39	49	47	53	52	37	47	40	32.84 - 34.85	33.84	0.62	0.16	1.84
Snout tip to second dorsal origin	94	82	92	86	76	83	74	72	63	82	79	88	87	60	76	67	53.73 - 57.34	55.81	0.84	0.21	1.51
Snout tip to anal fin origin	93	82	92	88	76	83	76	78	63	80	80	89	89	61	78	70	54.71 - 58.33	56.61	1.04	0.26.	1.84
Least depth of caudal peduncle	12	11	13	12	11	11	10	10	9	11	11	12	12	8	11	10	7.06 - 8.33 In Head length	7.72	0.35	0.09	4.47
Length of snout	16	14	17	16	15	16	14	14	12	15	16	17	18	12	15	13	36.84 - 42.86	39.27	2.05	0.51	5.21
Horizontal diameter of eye	10	9	11	9	10	9	9	9	7	9	10	11	11	7	10	7	21.88 - 25.64	24.12	1.13	0.28	4.68
Least width of interorbital	7	7	8	7	8	7	8	6	6	7	7	9	8	6	8	6	16.28 - 21.43	18.83	1.50	0.38	7.99
Sex	F	F	F	M	F	M	M	M	F	M	F	M	M	F	F	F					
Locality	MMM	MMM	RMM	PBN	MDL	KNL	PBN	RMM	RMM	RMM	MMM	MMM	MMM	DHI	DHI	DHI					

MMM=Mandapam; RMM=Rameswaram; PBN=Pamban; MDL=Mundal; MNL=Kundugal; DHI=Dhanushkudi

TABLE 4. Morphometric characters of *Sillago indica* Mckay, Dutt and Sujatha, 1985.
(all measurements in mm)

Characters	1	2	3	4	5	6	7	8	9	Range %	Mean %	Standard Deviation	Standard Error	Coefficient of variation
Standard length	150	152	125	141	144	165	144	193	152	In Standard length				
Greatest depth of body	28	32	24	27	30	32	30	41	28	18.42 - 21.24	19.86	1.11	1.59	5.59
Head length	42	44	36	41	41	48	41	56	43	28.00 - 29.09	28.69	0.39	0.13	1.37
Snout tip to ventral fin origin	45	46	39	43	43	51	44	59	46	29.86 - 31.20	30.46	0.42	0.14	1.25
Snout tip to spinous dorsal origin	50	51	43	47	49	55	48	65	52	33.33 - 34.40	33.69	0.42	0.14	1.25
Snout tip to second dorsal origin	81	86	68	79	76	94	76	108	82	52.76 - 56.97	54.83	1.60	0.53	2.91
Snout tip to anal fin origin	82	85	71	79	81	89	80	108	83	53.94 - 56.80	55.53	0.91	0.31	1.66
Least depth of caudal peduncle	11	12	9	10	12	12	12	15	11	7.09 - 8.33	7.61	0.49	0.16	6.42
										In Head length				
Length of snout	16	16	14	16	16	18	16	21	17	36.36 - 39.53	38.33	1.03	0.34	2.69
Horizontal diameter of eye	8	9	7	9	9	9	9	1	8	18.60 - 21.95	20.20	1.40	0.47	7.03
Least width of interorbital	8	8	7	8	8	10	8	11	9	18.18 - 20.93	19.62	0.84	0.28	4.28
Sex	M	F	M	F	F	F	M	F	M					
Locality	RMM	RMM	PBN	RMM	PBN	MMM	MMM	PBN	RMM					

RMM=Rameswaram; PBN=Pamban; MMM=Mandapam.

TABLE 5. Morphometric characters of *Sillago vincenti* Mckay, 1980.
(all measurements in mm)

Characters	1	2	3	4	5	Range %	Mean %	Standard deviation	Standard Error	Coefficient of variation
Standard length	211	216	204	217	186	In standard length				
Greatest depth of body	37	36	36	43	35	16.67 - 19.82	18.06	1.20	0.54	6.67
Head length	55	57	56	63	52	21.07 - 29.03	27.32	1.17	0.52	4.29
Snout tip to ventral fin origin	56	60	58	65	54	26.54 - 29.95	28.28	1.25	0.56	4.43
Snout tip to spinous dorsal origin	65	75	69	76	61	30.81 - 35.02	33.36	1.74	0.78	5.22
Snout tip to second dorsal origin	113	118	110	119	101	53.55 - 54.84	54.13	0.57	0.25	1.65
Snout tip to anal fin origin	115	120	111	116	102	53.46 - 55.56	54.44	0.75	0.33	1.38
Least depth of caudal peduncle	13	14	14	15	12	6.16 - 6.91	6.56	0.32	0.14	4.89
						In Head length				
Length of snout	22	24	24	28	24	40.00 - 46.15	43.11	2.33	1.04	5.41
Horizontal diameter of eye	9	11	11	14	10	16.36 - 22.22	19.35	2.08	0.93	10.74 *
Least width of interorbital	9	10	11	12	9	16.36 - 19.64	17.98	1.34	0.60	7.44
Sex	M	F	F	F	F					
Locality	PBN	PBN	PBN	PBN	MNL					

PBN=Pamban; MNL=Manoli

TABLE 6. Morphometric characters of *Sillago argentifasciata* Martin and Montalban, 1935.

(all measurements in mm)

Characters	1	2	3	4	5	6	7	Range %	Mean %	Standard Deviation	Standard Error	Coefficient of variation
Standard length	150	147	144	160	148	163	133	In Standard length				
Greatest depth of body	29	29	28	32	29	33	25	18.80 - 20.25	19.59	0.47	0.18	2.41
Head length	44	44	43	48	44	49	39	29.32 - 30.06	29.75	0.31	0.12	1.03
Snout tip to ventral fin origin	47	46	44	50	46	52	42	30.56 - 31.90	31.28	0.42	0.16	1.33
Snout tip to spinous dorsal origin	50	51	50	56	50	55	47	33.33 - 35.34	34.37	0.75	0.28	2.19
Snout tip to second dorsal origin	84	83	82	92	85	93	77	56.00 - 57.89	57.04	0.65	0.25	1.13
Snout tip to anal fin origin	84	85	84	91	84	94	78	56.00 - 58.65	57.44	0.94	0.35	1.64
Least depth of caudal peduncle	12	12	12	13	12	13	11	8.00 - 8.33	8.14	0.13	0.05	1.58
								In Head length				
Length of snout	17	17	18	19	18	21	15	38.46 - 42.86	40.14	1.76	0.66	4.38
Horizontal diameter of eye	12	13	12	14	12	14	11	27.27 - 29.55	28.28	0.88	0.33	3.12
Least width of interorbital	8	8	8	9	8	9	7	17.95 - 18.75	18.32	0.28	0.11	1.51
Sex	F	M	M	F	F	M	F					
Locality	RMM	RMM	PBN	PBN	RMM	PBN	RMM					

RMM=Rameswaram; PBN=Pamban

TABLE 7. Morphometric characters of *Sillago soringa* Dutta and Sujatha 1983.
(all measurements in mm)

Characters	1	2	3	4	5	Range %	Mean %	Standard Deviation	Standard Error	Coefficient of variation
Standard length	132	135	115	125	120	In standard length				
Greatest depth of body	23	24	20	23	23	17.39 - 19.17	18.03	0.76	0.34	4.19
Head length	37	38	32	36	34	24.83 - 28.80	28.20	0.42	0.19	1.49
Snout tip to ventral fin origin	40	41	36	38	37	30.30 - 31.30	30.64	0.42	0.19	1.38
Snout tip to spinous dorsal origin	45	47	40	39	42	31.20 - 35.00	33.98	1.59	0.71	4.68
Snout tip to second dorsal origin	74	78	67	71	70	56.06 - 58.33	57.45	0.99	0.44	1.72
Snout tip to anal fin origin	72	76	63	69	67	54.55 - 56.30	55.33	0.73	0.33	1.32
Least depth of caudal peduncle	9	9	8	8	8	6.40 - 6.96	6.70	0.21	0.09	3.11
						In head length				
Length of snout	14	15	12	14	13	37.50 39.47	38.39	0.80	0.36	2.07
Horizontal diameter of eye	9	10	8	10	10	24.32 - 29.41	26.57	2.07	0.92	7.79
Least width of interorbital	8	8	6	8	7	18.75 22.22	20.85	1.32	0.59	6.33
Sex	F	F	M	F	M					
Locality	RMM	PBN	MMM	PBN	RMM					

RMM=Rameswaram; PBN=Pamban; MMM=Mandapam.

TABLE 8. Meristic characters of sillaginid fishes collected during the present study. (Frequency of specimens is given)

SPECIES	DORSAL RAYS							ANAL RAYS									LATERAL LINE SCALES													
	17	18	19	20	21	22	23	16	17	18	19	20	21	22	23	24	62	63	64	65	66	67	68	69	70	71	72	73	74	
Sillago chondropus					4										4										3	1				
Sillago sihama					13	3								13	3									9	7					
Sillago indica					5	4							2	4	3										2	3			2	
Sillago Vincenti					1	4								3	1	1										3	1	1		
Sillago Argentifasciata	6	1						4	3								1	1	1	2	2		1							
Sillago soringa					5									5					1		1		3							

(Cont'd.....)

SPECIES	VERTEBRAE																							
	ABDOMINAL			MODIFIED								CAUDAL												
	12	13	14	3	4	5	6	7	8	9	10	10	11	12	13	14	15	16	17	18	19	20	21	22
Sillago chondropus		4																						4
Sillago sihama			16		4	8	4									4	8	4						
Sillago indica			9	8	1													1	8					
Sillago vincenti			5		2	1	2									2	1	2						
Sillago argentifasciata		7							4		3	3	4											
Sillago soringa		5				3	2								2		3							

TABLE 9. List of Sillaginid fishes occurring in Indian waters.

S. No.	Species	Authors
1.	*Sillago(Sillaginopodys) Chondropus**	(Bleeker, 1849)
2.	*Sillago (Sillago) Sihama**	(Forskal, 1775)
3.	*Sillago (Sillago) Intermedius*	Wongratana, 1977
4.	*Sillago (Parasillago) indica**	McKay, Dutt & Sujatha, 1985
5.	*Sillago (Parasillago) vincenti**	Mckay, 1980
6.	*Sillago (Parasillago) argentifasciata**	Martin & Montalban, 1935
7.	*Sillago(Parasillago) soringa**	Dutt & Sujath, 1983
8.	*Sillago(Parasillago) Lutea*	Mckay, 1985
9.	*Sillaginopsis panijus*	(Hamilton Buchanan, 1822)

* Species collected during the present study from Palk Bay and Gulf of Mannar

CHAPTER - IV

MATURATION AND SPAWNING

Maturation and spawning in teleost fishes have been extensively studied. In addition to the assessment of morphological changes taking place in gonads during their development, seasonal occurrence of fish in different stages of maturity, minimum size at maturity, fecundity, gonadosomatic index, sex-ratio etc. have been studied by a number of workers in different regions of the world.

General aspects of reproductive biology of several temperate and sub-tropical teleosts have been studied. A few noteworthy examples are: *Epinephelus* spp (Smith, 1961); *Trachurus trachurus* (Macer, 1974); *Fundulus heteroclitus* (Wallace and Selman, 1981); *Maena maena* (Sellami and Brusle, 1979); surf perches (Darling *et al.*, 1980); *Paralichthys dentatus* (Morse, 1981); *Gerres* sp. (Cyrus and Blaber, 1984)..

In fishes, reproduction, like any other physiological process, follows a cyclic pattern, the periodicity and timing of which are under the dual control of endogenous and exogenous factors. Detailed reviews on this subject have been given by deVlaming (1972a, 1974), Billard *et al.*, (1978), Scott(1979), Wootton (1982), Lam (1983) and Bye (1984).

Minimum size at first maturity of a number of teleost fishes have been determined. To mention a few, this aspect was studied in Albacore tuna (Otsu and Vehida, 1959), *Micropterus salmoides* (Kelly, 1962), *Lates calcarifer* (Davis, 1982) and *Melanogrammus aeglefinus* (Templeman *et al.*, 1978).

Bagenal (1957b, 1967, 1968, 1971, 1978) has carried out extensive work on fish fecundity. A perusal of the literature on fish fecundity shows that there has been some variations in its definition based on the stage of ova counted. The difficulty in accurate determination of fecundity in species where the ova are destined to be withdrawn from the ovaries in batches has been mentioned (Qasim, 1973). Macer (1974) has described a method of estimating fecundity in fishes where the ova development is asynchronous.

In Indian waters, the reproductive biology of a number of commercially exploited marine fishes has been investigated. To mention a few examples, reproduction in *Mugil* spp (Sarojini, 1957, 1958), *Sardinella longiceps* (Antony Raja, 1964) Ribbon fishes (James, 1967), *Harpodon nehereus* (Bapat, 1967) and *Rastrelliger kanagurta* (Nair and Rao, 1970), has been studied in detail. Seasonal cycle in the spawning of *Polydactylus indicus* (Karekar and Bal, 1960), *Lethrinus lentjan* (Toor, 1964), *Pampus chinensis* (Pati, 1979) and *Tachysurus dussumieri* (Vasudevappa and James, 1980) has been described.

General aspects of the reproductive biology of some western Pacific sillaginids have been investigated. Seasonal cycle in the spawning of *Sillago maculata* (Ogilby, 1903) *Sillago ciliata* (Cleland, 1947), *Sillago schomburgkii* (Thompson, 1957 d) *Sillago japonica* (Kashiwagi and Yamada, 1984) has been studied. Morton (1982) studied reproductive biology of *Sillaginodes punctatus*. Minimum size at first maturity in *Sillago ciliata* (Cleland, *op. cit.*), *S. schomburgkii* (Thompson, *op. cit.*) and *S. robusta* (Grant, 1965) was determined. Ueno and Fujita (1954) described the development of eggs of *Sillago sihama*.

In Indian waters, reproductive biology of *Sillago sihama* was studied at Karwar (Palekar and Bal, 1961), Netravathy and Gangolli estuaries (James *et al.*, 1976) and Mandapam (Radhakrishnan, 1957). Though these works have contributed useful information about reproduction in *Sillago*, a comprehensive account on all aspects of reproduction is lacking. Further, only since 1980 it has been established that 9 nominal species of the family Sillaginidae occur in Indian waters (Dutt and Sujatha, 1983), and that some of them have very similar external appearance, liable to be mistaken as a single species. In the present study, due importance was given to proper identification of the species. *Sillago sihama*, being the most dominant sillaginid in the area the present study, was taken up to investigate different aspects of maturation and spawning.

OBSERVATIONS

Reproductive organs

Ovary

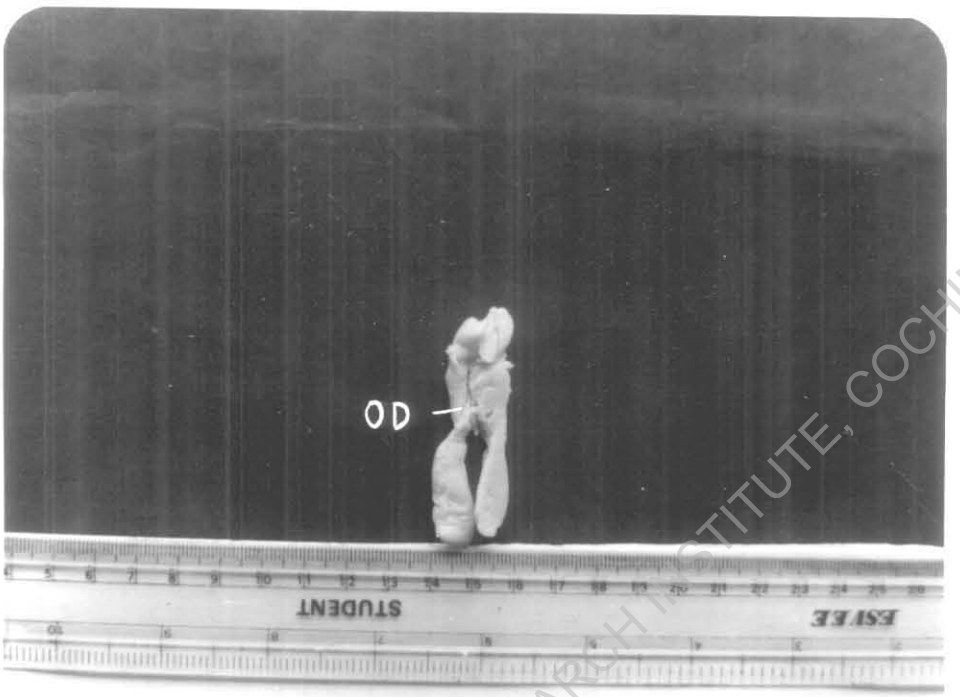
The female reproductive system of *Sillago sihama*, consists of a pair of ovaries, oviducts and a common ovarian duct (Plate VI, Fig. 1). They lie ventral to the swimbladder in the body cavity and are attached to the dorsal body wall by a thick mesovarium and to the viscera and swimbladder by thin mesenteries.

Each ovary contains a central cavity, the ovocoel, which continues ventrally and backward into a short oviduct, a few millimeters in length. The two oviducts join together to form a common tube, which opens to the exterior through a urinogenital pore which is situated just posterior to the

PLATE VI.

Fig. 1. Reproductive organs of female *S. sihama*.
OD-Oviduct.

Fig.2. Reproductive organs of male *S. sihama*.
VD-Vas deferens.



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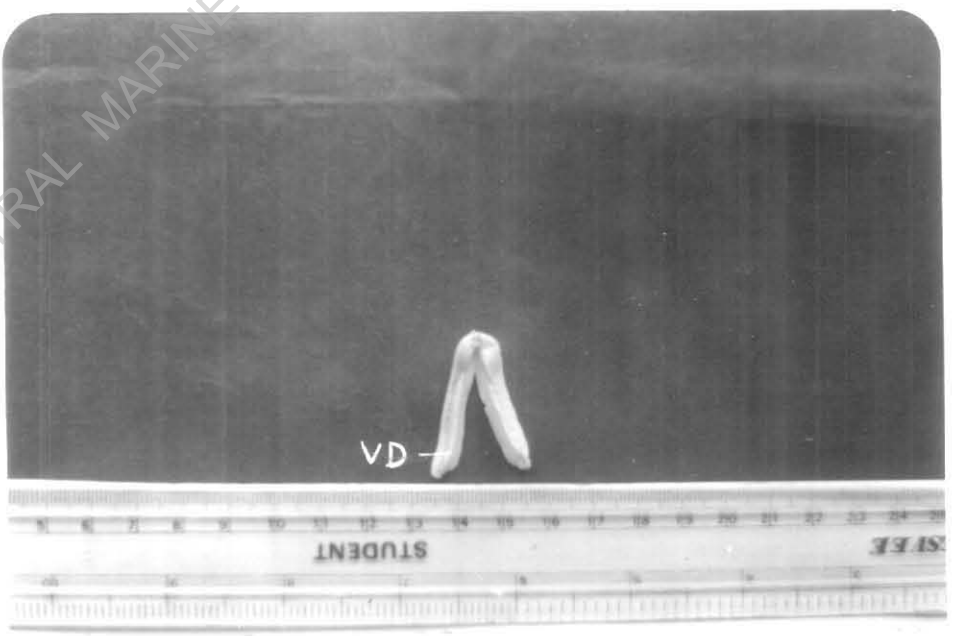


2

anal opening. Though the ovaries of *S. sihama* are usually asymmetry some cases of asymmetry were noticed during the present study.

Testis

The male reproductive system consists of a pair of elongated testes, vasa deferentia and a common sperm duct (Plate VI, Fig. 2). The testes appear to be laterally compressed, with the two lobes being almost uniform in width, except at the posterior ends, where they are found to taper slightly. The testes are attached to the roof of the peritoneal cavity by means of connective tissue strands known as mesorchium. The mesorchium also supports the genital blood vessel.

The vas deferens runs throughout the entire length of each of the testis along its inner lateral side. Posteriorly the two vasa deferentia are united to form a common sperm duct, which is covered by a connective tissue sheath formed by the mesorchium.

The two lobes of the testes are more or less of the same size, though cases of asymmetry are not uncommon. In one extreme case of asymmetry, the left lobe was flat and creamy white, extending almost the entire body cavity, while the right lobe was very short and hard to detect.

No accessory reproductive organs were found in association with either female or male reproductive system in *Sillago sihama* .

Classification of maturity stages

Female

Based on the size, colour and appearance, the ova were classified into four stages, namely Immature ova, Maturing ova, Mature ova and Ripe ova (Plate VII).

PLATE VII.

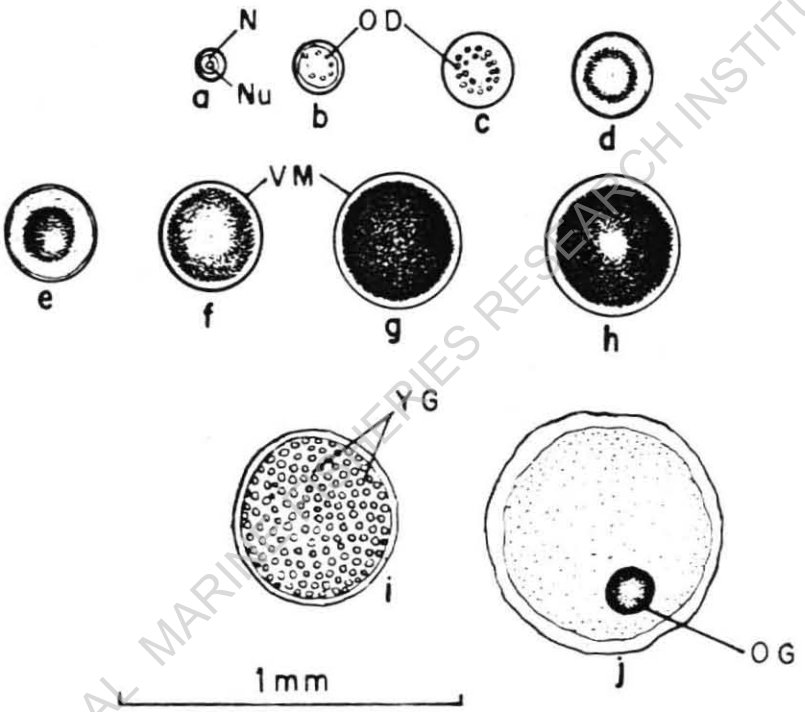
Different stages of intra-ovarian ova of *S. sihama*

a-d: Immature ova; e-f: Maturing ova; i-Mature ovum;

j-Ripe ovum; N-Nucleus; Nu-Nucleolus; OD-Oil droplets;

VM-vitelline membrane; YG-Yolk granules; OG-Oil globule.

PLATE VII



Immature ova: Transparent and tiny ova with a prominent central nucleus and a thin protoplasmic layer. Diameter of the immature ova varies between 0.0167 and 0.217 mm. Ova measuring upto 0.117 mm have no indication of yolk accumulation, while those above 0.133 mm have tiny vacuoles of varying number in their ooplasm clustered around the large nucleus.

Maturing ova: These ova are characterised by varying degree of yolk accumulation in their cytoplasm as evidenced by their opaqueness in fresh condition. Their diameter ranges from 0.233 to 0.4 mm. Ova of 0.233-0.255 mm have a dark perinuclear zone of yolk. This zone increases in width as the ova develop further and, at 0.3 mm, the ova will have their ooplasm filled with yolk except for a prominent transparent central zone and a clear perivitelline space.

Mature ova: The diameter of mature ova ranges between 0.417 and 0.517 mm. Those ova measuring upto about 0.467 mm have a dark ooplasm with a central translucent zone. The larger mature ova are filled with transparent yolk globules. These ova are still contained within the follicles.

Ripe ova: These are large and transparent with a prominent oil globule. They are freed from the follicular envelope. The diameter of the ripe ova varies from 0.533 to 0.783 mm and that of the oil globule ranges between 0.15 and 0.167 mm.

Maturity condition of female fish has been classified into 5 stages based on the general appearance of ovaries and the occurrence of various ova classes described above. The reproductive stages are as follows:

Stage -I Immature fish: The ovaries are thin, short and glassy in appearance. They do not extend more than half the body cavity length and

weigh between 9 and 527 mg. The ovary of the immature fish contains only the immature group of ova. The maximum total length of the immature female fish was 185 mm in the present study.

Stage-II Maturing fish: The ovaries are opaque and creamy yellow in colour. They occupy about 2/3 of the body cavity length and weigh between 0.18 and 4.16 g. The ovary of the maturing fish contains about 52% of immature ova, 36% maturing ova and 12% mature ova. Total length of the maturing female fish varies from 136 to 226 mm.

State-III Mature fish: The ovaries are reddish yellow in colour and extends about 3/4 in body cavity length. The ovaries weigh between 0.343 and 3.641 g and contain about 41% of immature ova, 34% maturing ova and 25% mature ova. Total length of the mature female fish ranges between 139 and 232 mm.

Stage-IV Ripe fish: The ovaries are yellow to amber coloured and fill the entire body cavity. Large translucent ova are visible through the ovarian membrane and the blood vessels are engorged. Ripe ova can be extruded from genital papilla by slight pressure on abdomen. The ovaries weigh between 0.492 and 6.103 g and contain about 40% immature ova, 27% maturing ova, 16% mature ova and 17% ripe ova. Spawning is imminent at this stage. Total length of the ripe female fish varies from 147 to 245 mm.

Stage -V Partially spent fish: The ovaries are slightly flaccid and reddish yellow in colour. They extend about $\frac{1}{2}$ the body cavity length. The ovarian membrane is thickened, more opaque than in the previous stage, with vascular engorgement. The partially spent ovary contains

about 44% immature ova, 38% maturing ova, 13% mature ova and 5% residual ripe ova. The ovaries weigh between 0.45 and 0.7 g. Total length of Partially spent female fish varies from 180 to 217 mm.

Male

Based on the size, shape and colour of the testes, the male has been classified into 5 maturity stages, namely Immature, Maturing, Mature, Oozing and Partially spent.

Stage I Immature fish: Testes are thin, semi-transparent, thread-like organs extending less than $\frac{1}{2}$ in the body cavity length. The testes weigh between 4 and 114 mg. The maximum length (total) of the immature male fish collected during the present study was 164 mm.

Stage II Maturing fish: Testes are moderately thick, flattened and white in colour. They are opaque with smooth surface and extend about 2/3 in the body cavity length and weigh between 45 and 153 mg. On the inner lateral surface of the testis, a furrow becomes discerinible at this stage due to the appearance of the vas deferens. Total length of the maturing male fish ranges from 132 to 185 mm.

Stage III Mature fish: Testes are flat, well-developed and creamy white coloured organs in this stage. They extend more than 2/3 in body cavity length and weigh between 144 and 490 mg. Each testis has a vas deferens running throughout its entire length. A small amount of milt oozes when pressure is applied to the abdomen. Total length of mature male fish varies from 139 to 205 mm.

Stage IV Oozing fish: Testes are very thick, flat, turgid and creamy white in colour. They fill the entire body cavity and weigh between 0.522

and 1.45 g. Milt oozes out freely with slight pressure applied on the abdomen. Copious amount of milt oozes out from the cut ends of the testis. Total length of Oozing male fish varies from 163 to 209 mm.

Stage V Partially spent fish: Testes are shrunken, somewhat flaccid with the surface thrown into folds. They are white in color. Compared to the previous stage, less quantity of milt oozes out on application of pressure to the abdomen. The testes weigh between 200 and 350 mg. Total length of the partially spent male fish varies from 175 to 195 mm.

Development of ova to maturty

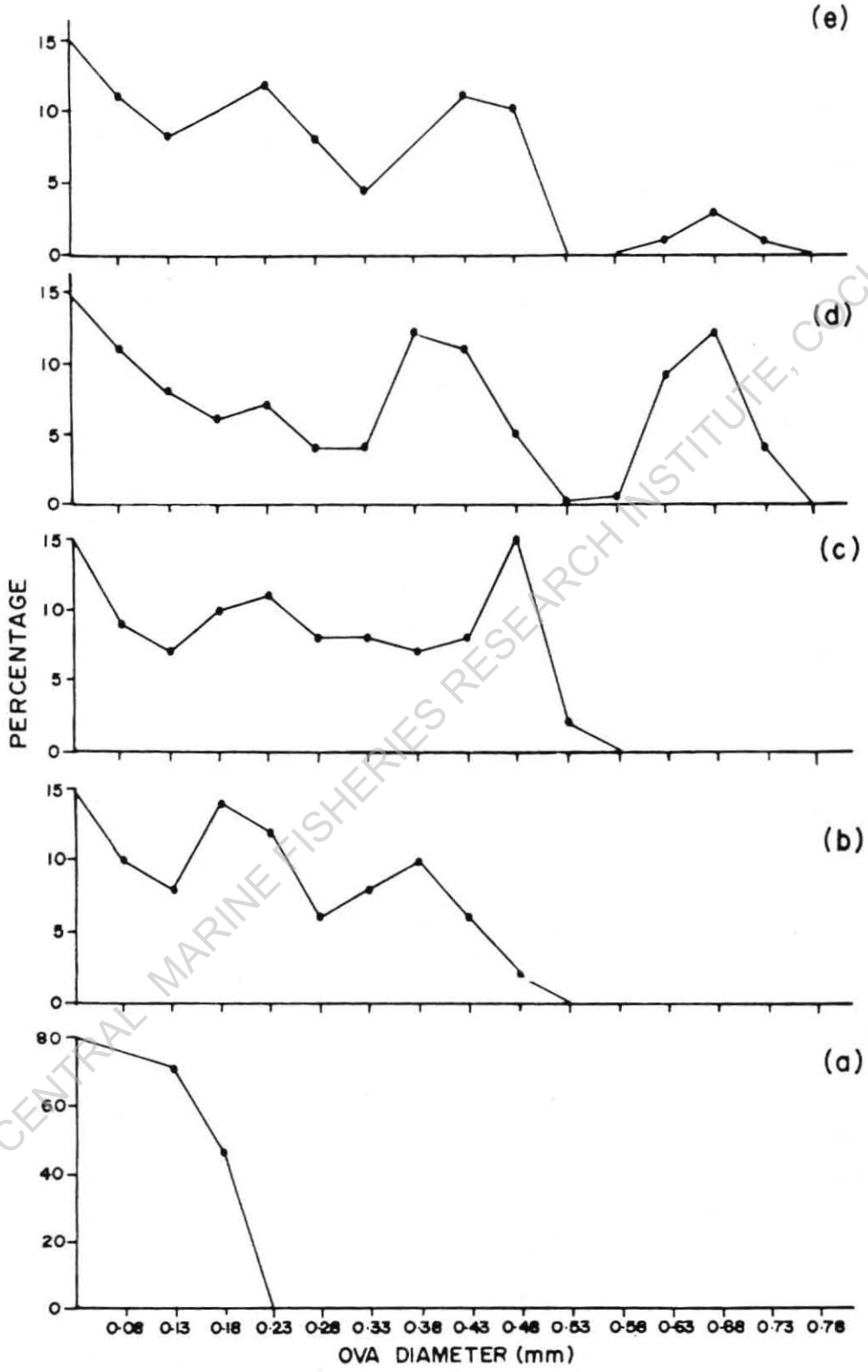
Size distribution of ova in the five maturity stages of ovary is given in Plate VIII. The observation are based on 16 ovaries for each stage of maturity. In the immature ovary, majority of the ova are in the size range 0.017-0.13 mm and a few measure up to 0.22 mm. The immature stock of ova are found in all the subsequent stages of ovary. The polygon of maturing ovary (Stage II) indicates withdrawal of a batch of ova with a modal size of 0.18 mm. Another distinct mode is formed at 0.38 mm. The latter represent the maturing stock, in which vitellogenesis has commenced. In the mature ovary (Stage III), the mature group of ova is well demarcated from the maturing and immature groups by a mode at 0.48 mm. In this stage the maturing group of ova are forming a mode at 0.23 mm. The ripe ovary (Stage IV) contains, apart from immature ova, maturing ova (modes at 0.23 mm and 0.38 mm), mature ova and ripe ova (mode at 0.68 mm). Actually the mature group of ova which formed a mode at 0.48 mm in stage III, has further developed to form the ripe ova which formed a mode at 0.68 mm in stage IV. The maturing and mature groups of ova pass in

PLATE VIII.

The ova diameter frequency polygons of different maturity stages of *S. sihama*

a-Immature stage; b-Maturing stage; c-Mature stage; d-Ripe stage; e-Partially spent stage.

PLATE VIII



succession to advanced stages of maturity, when fresh batches of maturing ova arising from the immature stock takes their place. It may be seen in the polygon of ripe ovary that, the ripe ova forms a distinct group with a clear mode. At any single spawning only this group may be expected to be ovulated and spawned. The ova diameter Polygon of partially spent (Stage V) ovary shows, apart from immature, maturing and mature ova, few residual ripe ova forming a mode at 0.68mm. These ova are almost transparent with oil globules, but most of them are shrunken and distorted in shape. Presence of residual ova constitutes good evidence that spawning has occurred.

Spawning period

A total of 1184 fishes during April 1984 to March 1985 and 1240 fishes during April 1985 to March 1986 were examined to study the percentage occurrence of different stages of maturity in different months. The data are presented in Tables 10 to 13.

Female

In April 1984, stage I to IV occurred, with stage II dominating over the others. In May, all maturity stages of female fish were present with stage I and stage II being more than the rest. In June, all maturity stages were available, with stage II was dominating. In July, fish of all stages from I to V were recorded with stage IV being slightly dominant than the stages II and III. In August, stages I to IV occurred, with stage III being dominant. In September and October, all maturity stages were present. In November, stages II to V were recorded with stage IV forming 50% of all the stages. In December, all maturity stages were present, stage II being dominant. In

TABLE 10. Monthly occurrence of different maturity stages of female *Sillago sihama* during April, 1984-March, 1985

Months	No. of fish examined	Maturity Stages (Percentage)				
		I	II	III	IV	V
April, 1984	70	30.00	40.00	25.00	5.00	-
May	48	43.75	39.58	10.43	4.17	2.08
June	56	23.21	60.71	5.36	7.14	1.79
July	44	9.09	27.27	27.27	29.55	6.82
August	40	5.00	35.00	40.00	20.00	-
September	48	20.83	39.58	16.67	16.67	6.25
October	40	10.00	15.00	40.00	30.00	5.00
November	64	-	21.88	21.88	50.00	6.24
December	54	22.22	46.30	11.11	16.67	3.70
January,1985	54	-	44.44	33.33	20.37	1.85
February	48	31.25	50.00	6.25	12.50	-
March	60	50.00	30.00	20.00	-	-

TABLE 11. Monthly occurrence of different maturity stages of female *Sillago Sihama* during April 1985 - March 1986.

Months	No. of fish Examined	Marutiry stages (Percentage)				
		I	II	III	IV	V
April ,1985	70	20.00	50.00	30.00	-	-
May	60	38.33	35.00	16.67	5.00	5.00
June	50	28.00	60.00	6.00	6.00	-
July	50	20.00	18.00	24.00	34.00	4.00
August	50	18.00	20.00	36.00	24.00	2.00
September	54	14.81	40.74	20.37	20.37	3.70
October	64	15.63	15.63	29.69	35.94	4.69
November	56	-	25.00	28.57	39.29	5.36
December	42	21.43	30.95	26.19	21.43	-
January, 1986	44	10.00	50.00	15.00	20.00	5.00
February	50	16.00	62.00	6.00	16.00	-
March	58	32.76	32.76	34.48	-	-

TABLE 12. Monthly occurrence of different maturity stages of male *Sillago sihama* During April 1984 - March 1985

Months	No. of fish examined	Maturity stages (Percentage)				
		I	II	III	IV	V
April, 1984	50	20.00	64.00	16.00	-	-
May	64	20.31	29.69	29.69	18.75	1.56
June	42	-	59.52	16.67	21.43	2.38
July	36	-	25.00	63.89	11.11	-
August	24	4.17	25.00	54.17	16.67	-
September	44	-	22.93	63.64	13.64	-
October	32	-	15.63	50.00	25.00	9.38
November	40	-	20.00	40.00	35.00	5.00
December	60	20.00	40.00	13.33	25.00	1.67
January,1985	30	20.00	40.00	20.00	16.67	3.33
February	56	12.50	16.07	48.21	23.21	-
March	80	-	30.00	46.25	23.75	-

TABLE 13. Monthly occurrence of different maturity stages of male *Sillago sihama* During April 1985 - March 1986

Months	No. of fish Examined	Marurity stages (Percentage)				
		I	II	III	IV	V
April, 1985	68	19.12	60.29	19.12	1.42	-
May	64	18.75	35.94	35.94	7.81	1.56
June	34	8.82	47.06	38.24	5.85	-
July	48	10.42	25.00	50.00	14.58	-
August	30	10.00	30.00	33.33	26.67	-
September	46	10.87	19.57	47.83	17.39	4.34
October	58	5.17	10.34	43.10	36.21	5.18
November	40	-	15.00	35.00	42.50	7.50
December	42	16.67	35.71	16.67	30.95	-
January, 1986	36	-	44.44	25.00	27.78	2.78
February	52	17.31	-	50.00	32.69	-
March	82	-	31.71	63.41	4.88	-

January 1985, stage II to V occurred, with stage II being dominant. In February except stage V, all other stages were recorded, while in March only stage I to III occurred in the samples (Plate IX).

In April 1985, stage I to III occurred, with stage II being dominant. In May, all maturity stages were present, with stage I and II being more than the rest. In June, all stages of maturity except Stage V were recorded. From July to October, all maturity stages were present in the samples, with the advanced maturity stages being more in percentage. In November, as in the previous year, stage IV was most dominant, while stage I was absent. In December, except stage V all other stages of maturity occurred. In January 1986, all stages of maturity were available. In February, stages I to IV were recorded, with stage II being dominant. In March, only stages I to III were present, as was observed during the previous year.

A comparison of the above data on the maturity of *S. sihama* over two successive years indicates that fish of various stages of maturity occur in most of the months. However, it is evident from these data that fish in stages IV and V were recorded during the period from May to February. In both the years, maximum number of female fish in Ripe stage was present in November. It can be thus inferred that *S. sihama* has a prolonged breeding season extending from about July to February, with peak spawning activity during July to November period.

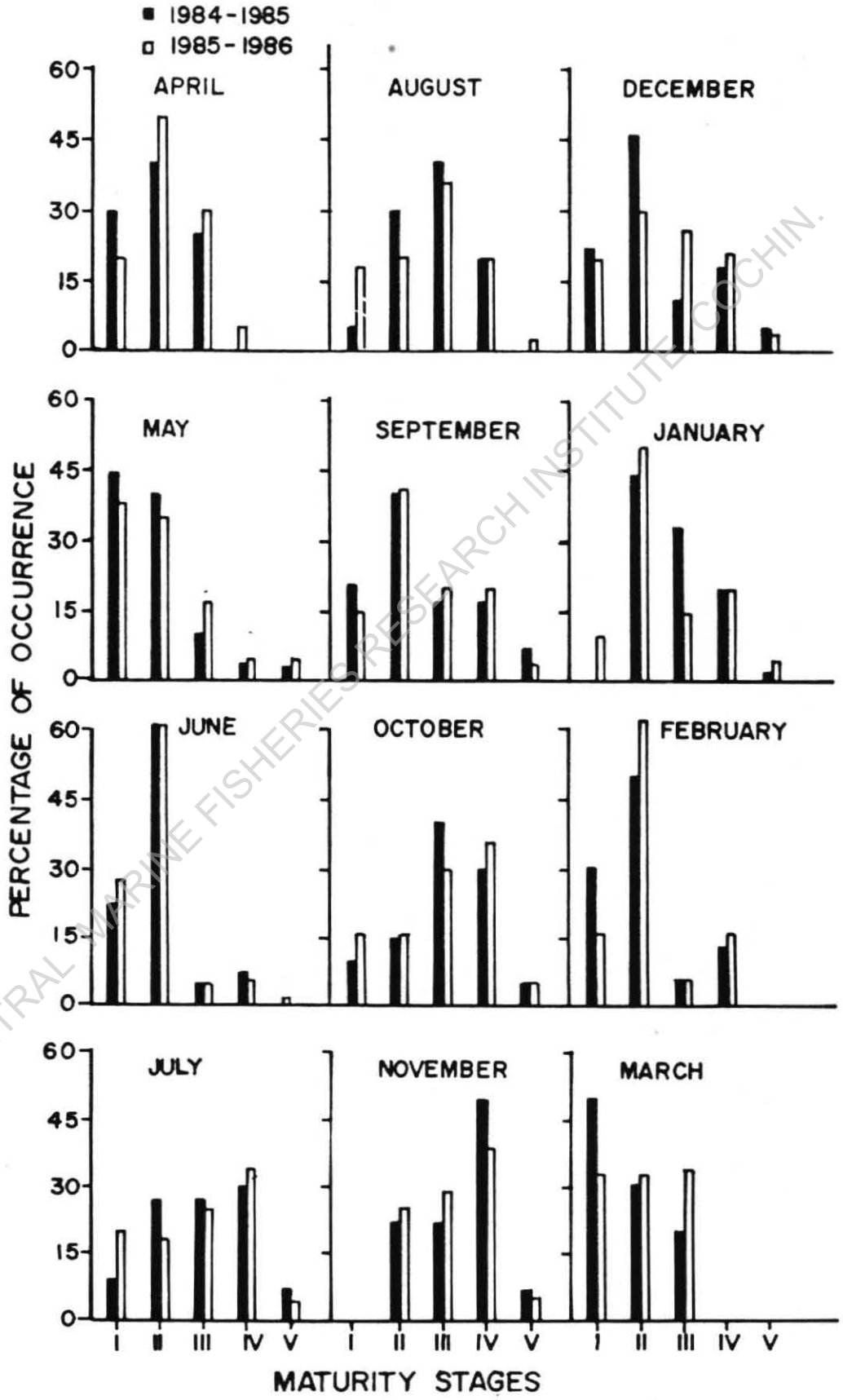
Male

In April 1984, stages I to III occurred, with stage II being most dominant. In May, all stages were present, while in June, except stage I all other stages were recorded. In July, stages II to IV occurred. In August, all other maturity stages except stage V were present, with stage III being dominant. In September,

PLATE IX

Percentage occurrence of female *S. sihama* during April 1984-March 1985 and April 1985-March 1986 periods.

PLATE IX



except stages I and V other stages were stages were present. In December and January 1985, all maturity stages were present, with stage II being more than the others. In February, stages I to IV occurred. In March, except stages I and V others were recorded, with stage III being dominant (Plate X).

During the following year, April 1985 to March 1986, an almost similar pattern of occurrence of male fish in different maturity stages was noticed. In April, stages I to IV occurred, with stage II being most dominant. In May all maturity stages were present, while in June, July and August, except stage V all other stages were recorded. In July, stage III formed about 50% of the total. In September and October all maturity stages were available. In November, except stage I other stages were recorded. In December stages I to IV were recorded, with stage II being most dominant. In January 1986, except stage I, all other stages were available, with stage II being dominant. In February, Stages I, III and IV occurred and in March, stages II to IV were present.

A perusal of the above data indicates that males in stages IV and V were present in most of the months, with greater occurrence during June - February period. As in the case of the females, maximum number of males in stage IV and V were recorded in November.

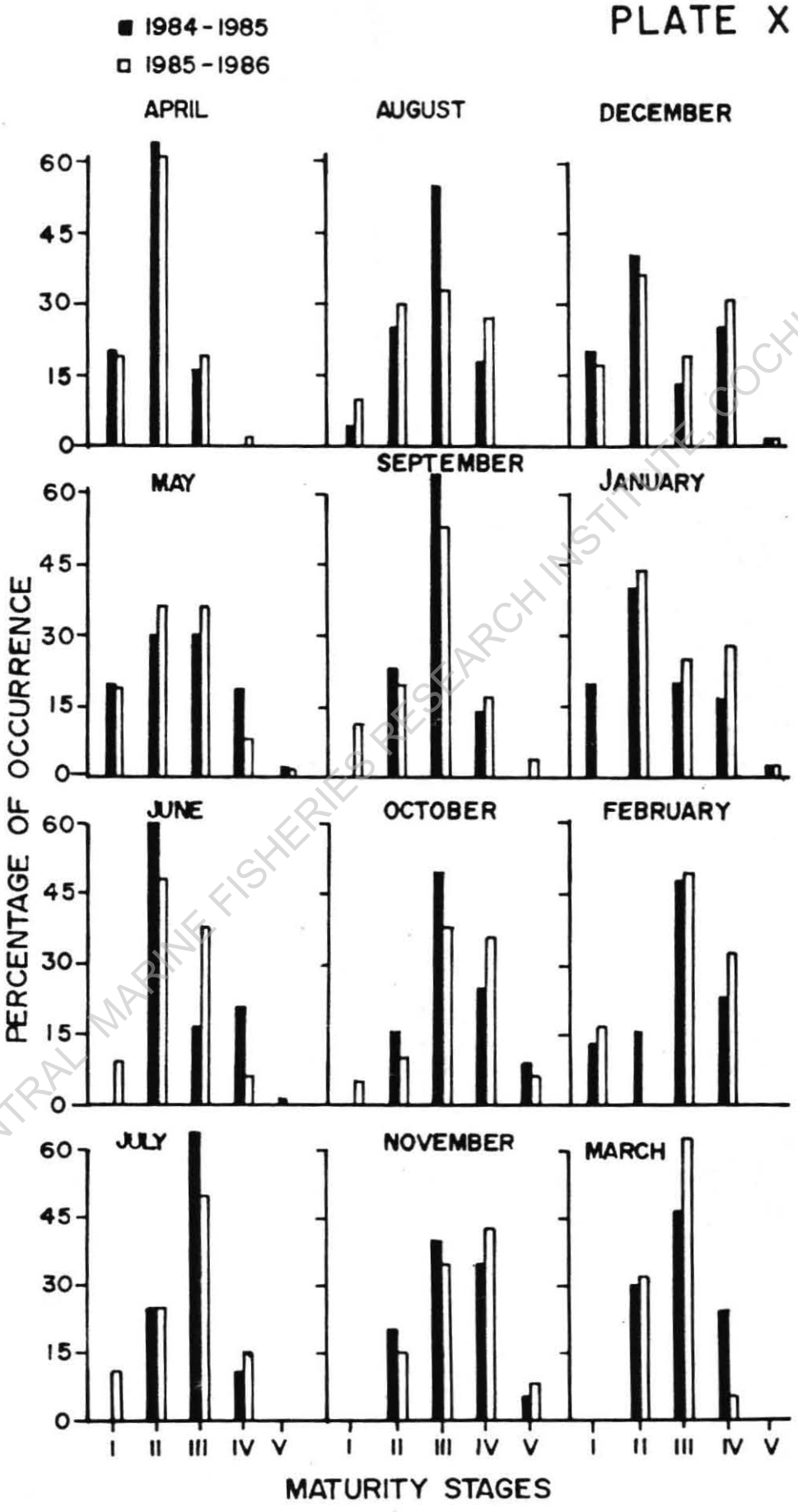
Gonadosomatic index

Gonadosomatic index is used to monitor breeding activity in fish. The mean Gonadosomatic index (GSI) values of different maturity stages of females were: Stage I - 0.33; stage II - 1.84; stage III - 2.21; stage IV - 3.40, stage V - 1.01. In males the mean GSI values were: Stage I - 0.08; stage II - 0.33; stage III - 0.72; stage IV - 1.33; stage V - 0.25.

PLATE X.

Percentage occurrence of male *S. sihama* during April 1984-March 1985 and April 1985-March 1986 periods.

PLATE X



The monthly mean GSI values for females and males are given in Tables 14 and 15 and depicted in Plate XI Figs. 1 & 2. Only speciemens above 139 mm in total length were taken for determining the monthly mean GSI values, since this was the minuimum size at which an individual fish (both sexes) was found to be in mature condition. It may be seen that during both the years, high GSI values have been noticed during the period from July to January, with maximum value in November in both sexes. This may indicate that during July to January, vigorous gonadal activity takes place in this fish.

However, there is one problem associated with utilizing mean GSI values in a prolonged breeder like *S. sihama* High standard deviations are observed while calculating mean GSI (Tables 14 and 15). This indicate that there is considerable variation in gonadal activity in each month. It is therefore questionable if the mean GSI reflects the gonadal activity of the majority of the population. Hence it is important to determine if this problem affects the conclusion drawn from the cycle of GSI values. In order to investigate this problem, the data on the occurrence of Stage IV (Ripe) fishes during different months may be consulted (Plates IX & X). These data clearly indicate that the high percentage of Stage IV during different months is coinciding with the high mean GSI values of both sexes. Similarly, the period of high gonadal quiescence, when a large proportion of the population is in immature conditions, coincides with the period of low mean GSI values during March to May.

PLATE XI

Fig. 1. Monthly mean gonadosomatic index values of *S. sihama* during April 1984-March 1985.

Fig.2. Monthly mean gonadosomatic index values of *S. sihama* during April 1985-March 1986.

PLATE XI

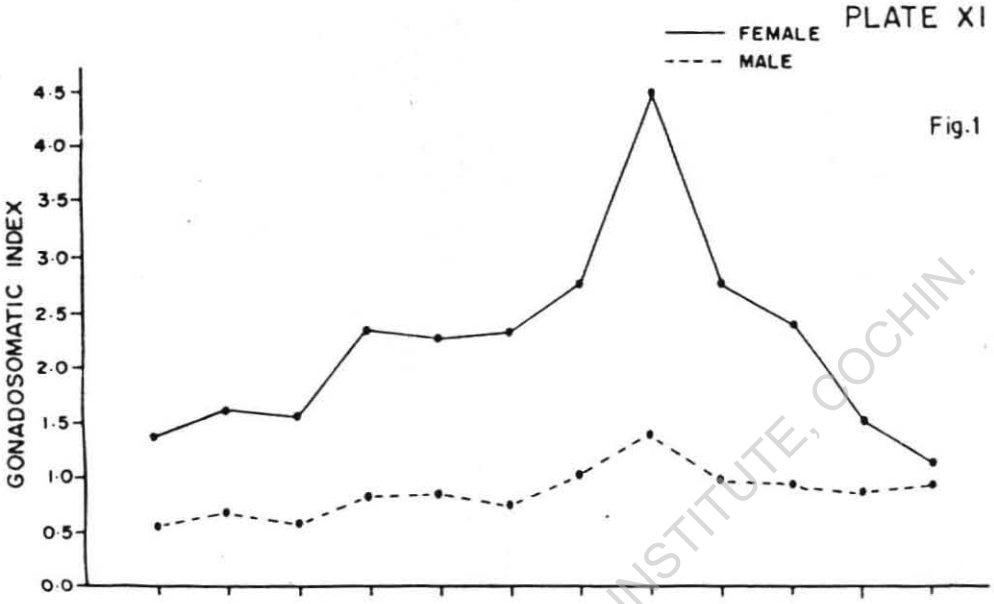


Fig.1

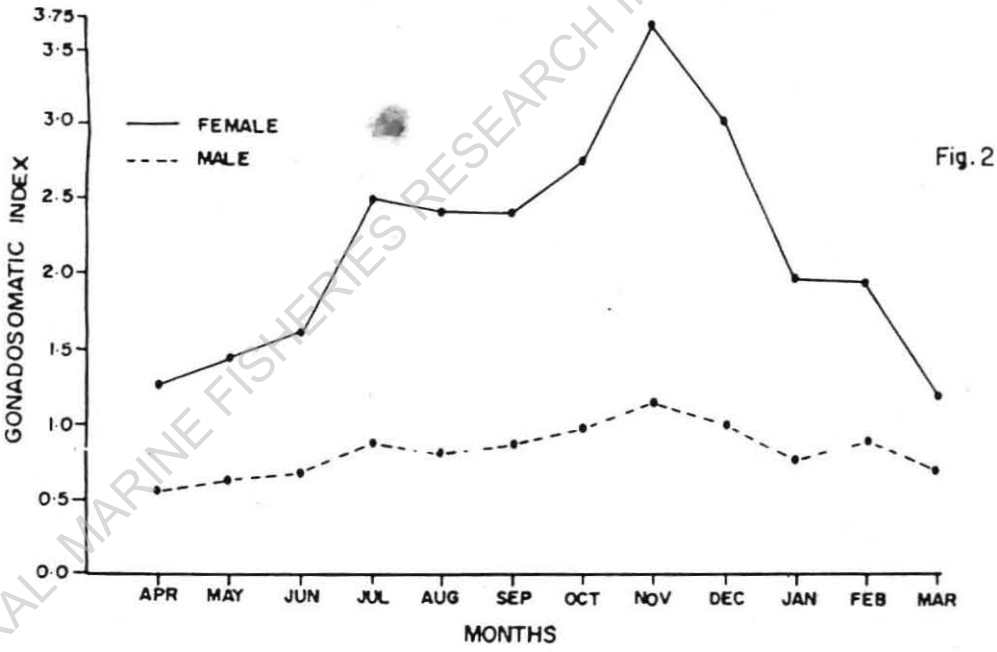


Fig.2

TABLE 14. Monthly Gonadosomatic Index Values of female *Sillago sihama* (± S.D.)

Months	April 1984 - March 1985	April 1985 - March 1986
April	1.33 ± 0.80	1.25 ± 0.71
May	1.59 ± 1.17	1.42 ± 0.80
June	1.50 ± 1.20	1.60 ± 0.80
July	2.30 ± 1.30	2.50 ± 1.17
August	2.22 ± 1.04	2.40 ± 1.47
September	2.29 ± 1.10	2.39 ± 1.15
October	2.73 ± 1.50	2.80 ± 1.45
November	4.45 ± 2.37	3.68 ± 1.50
December	2.70 ± 0.47	3.01 ± 1.50
January	2.38 ± 1.04	1.98 ± 1.70
February	1.50 ± 1.50	1.94 ± 1.65
March	1.12 ± 0.70	1.21 ± 0.71

TABLE 15. Monthly Gonadosomatic Index values of Male *Sillago sihama* (± S.D.)

Months	April 1984 - March 1985	April 1985 - March 1986
April	0.53 ± 0.40	0.55 ± 0.40
May	0.66 ± 0.30	0.61 ± 0.30
June	0.58 ± 0.45	0.68 ± 0.50
July	0.80 ± 0.45	0.88 ± 0.50
August	0.82 ± 0.60	0.75 ± 0.40
September	0.74 ± 0.40	0.88 ± 0.60
October	0.99 ± 0.70	0.98 ± 0.60
November	1.37 ± 0.70	1.15 ± 0.70
December	0.98 ± 0.70	1.01 ± 0.80
January	0.93 ± 0.71	0.75 ± 0.50
February	0.88 ± 0.64	0.90 ± 0.70
March	0.92 ± 0.70	0.69 ± 0.40

Size at first maturity

To determine the minimum size at first maturity of *S. sihama*, 392 females and 322 males during the period from July 1984 to February 1985 and 410 females and 344 males during the period from July 1985 to February 1986 were examined.

Fish were grouped sex-wise into 10 mm size groups and the percentage of fish in various stages of maturity in the size groups was calculated. For the purpose of calculating size at first maturity, fish belonging to stages III and IV have been grouped under mature fish. The details are presented in Tables 16 - 19.

From Table 16, it could be seen that during July 1984 to February 1985, up to 129 mm (total length), all the female fish were in the immature stage. From 130 mm onwards they pass into stage II and a few of them (16%) were found to be in the mature stage. In 170-179 mm size group, about 50% were in stage II and 41% in the mature stage. The Partially spent females were recorded for the first time in this group. In the next size group, namely 180-189 mm, as many as 53.33% of the fish were found mature. In the 190-199 mm size group, the percentage of fish in stage II were much less and 63.33% were in the mature group. From this size onwards, the percentage of mature fish gradually increased and all of them were mature in 220-229 mm size group. (Plate XII Fig. 3).

As may be seen from Table - 18, all the males were immature upto 129 mm. In 130-139 mm size group, 90% were immature, while a few (about 7%) in stage II and very few (about 3%) in mature stage. In the 150-159 mm size

TABLE 16 Percentage occurrence of different maturity stages of female *Sillago Sihama* in various size groups (July 1984 - February 1985)

Size groups (TL mm)	No. of Fish examined	Percentage of Maturity stages				
		I	II	III	IV	V
100 - 109	2	100.00	-	-	-	-
110 - 119	6	100.00	-	-	-	-
120 - 129	8	100.00	-	-	-	-
130 - 139	25	20.00	64.00	16.00	-	-
140 - 149	30	10.00	66.67	20.00	3.33	-
150 - 159	35	5.71	62.86	22.86	8.57	-
160 - 169	75	5.33	56.00	20.00	17.33	-
170 - 179	95	3.16	49.47	30.53	10.33	9.47
180 - 189	60	-	36.67	20.00	33.33	10.00
190 - 199	30	-	26.07	23.33	40.00	10.00
200 - 209	17	-	11.76	23.53	58.82	5.88
210 - 219	5	-	20.00	40.00	40.00	-
220 - 229	2	-	-	50.00	50.00	-
230 - 239	2	-	-	50.00	50.00	
240 - 249	2	-	-	50.00	50.00	-

TABLE 17. Percentage occurrence of different maturity stages of female *Sillago sihama* in various size groups (July 1985 - February 1986)

Size Groups (TL mm)	No. of fish examined	Percentage of Maturity Stages				
		I	II	III	IV	V
100 - 109	2	100.00	-	-	-	-
110 - 119	10	100.00	-	-	-	-
120 - 129	15	100.00	-	-	-	-
130 - 139	35	31.43	54.29	17.14	-	-
140 - 149	35	28.57	42.86	20.00	8.57	-
150 - 159	60	15.00	48.33	30.00	6.67	-
160 - 169	60	13.33	48.33	10.00	28.33	-
170 - 179	60	10.00	33.33	20.00	30.00	5.00
180 - 189	50	-	30.00	25.00	35.00	10.00
190 - 199	40	-	17.50	25.00	50.00	7.50
200 - 209	35		2.86	40.00	45.71	11.43
210 - 219	6	-	16.67	50.00	33.33	-
220 - 229	11	-			100.00	-
230 - 239	1	-			100.00	-
240 - 249	1	-			100.00	-

TABLE 18. Percentage occurrence of different maturity stages of male *Sillago sihama* in various size groups (July 1984 - February 1985)

Size Groups (TL mm)	No. of fish Examined	Percentage of maturity stages				
		I	II	III	IV	V
100 - 109	2	100.00	-	-	-	-
110 - 119	6	100.00	-	-	-	-
120 - 129	15	100.00	-	-	-	-
130 - 139	30	90.00	6.67	3.33	-	-
140 - 149	35	34.29	51.43	14.28	-	-
150 - 159	40	15.00	37.50	35.00	12.50	-
160 - 169	95	-	35.79	30.50	30.53	4.21
170 - 179	55	-	16.36	40.00	40.00	3.64
180 - 189	25	-	-	40.00	50.00	10.00
190 - 199	10	-	-	30.00	70.00	-
200 - 209	9	-	-	-	100.00	-
210 - 219	5	-	-	-	100.00	-

TABLE 19. Percentage occurrence of different maturity stages of male *Sillago sihama* in various size groups (July 1985 - February 1986)

Size groups (TL mm)	No. of fish Examined	Percentage of maturity stages				
		I	II	III	IV	V
100 - 109	4	100.00	-	-	-	-
110 - 119	6	100.00	-	-	-	-
120 - 129	15	100.00	-	-	-	-
130 - 139	30	76.67	20.00	3.33	-	-
140 - 149	45	44.44	26.67	28.89	-	-
150 - 159	60	20.00	40.00	20.00	20.00	-
160 - 169	96	-	39.58	20.83	39.59	-
170 - 179	50	-	18.00	40.00	40.00	2.00
180 - 189	30	-	-	46.67	46.67	6.67
190 - 199	4	-	-	25.00	75.00	-
200 - 209	4	-	-	-	100.00	-
210 - 219	2	-	-	-	100.00	-

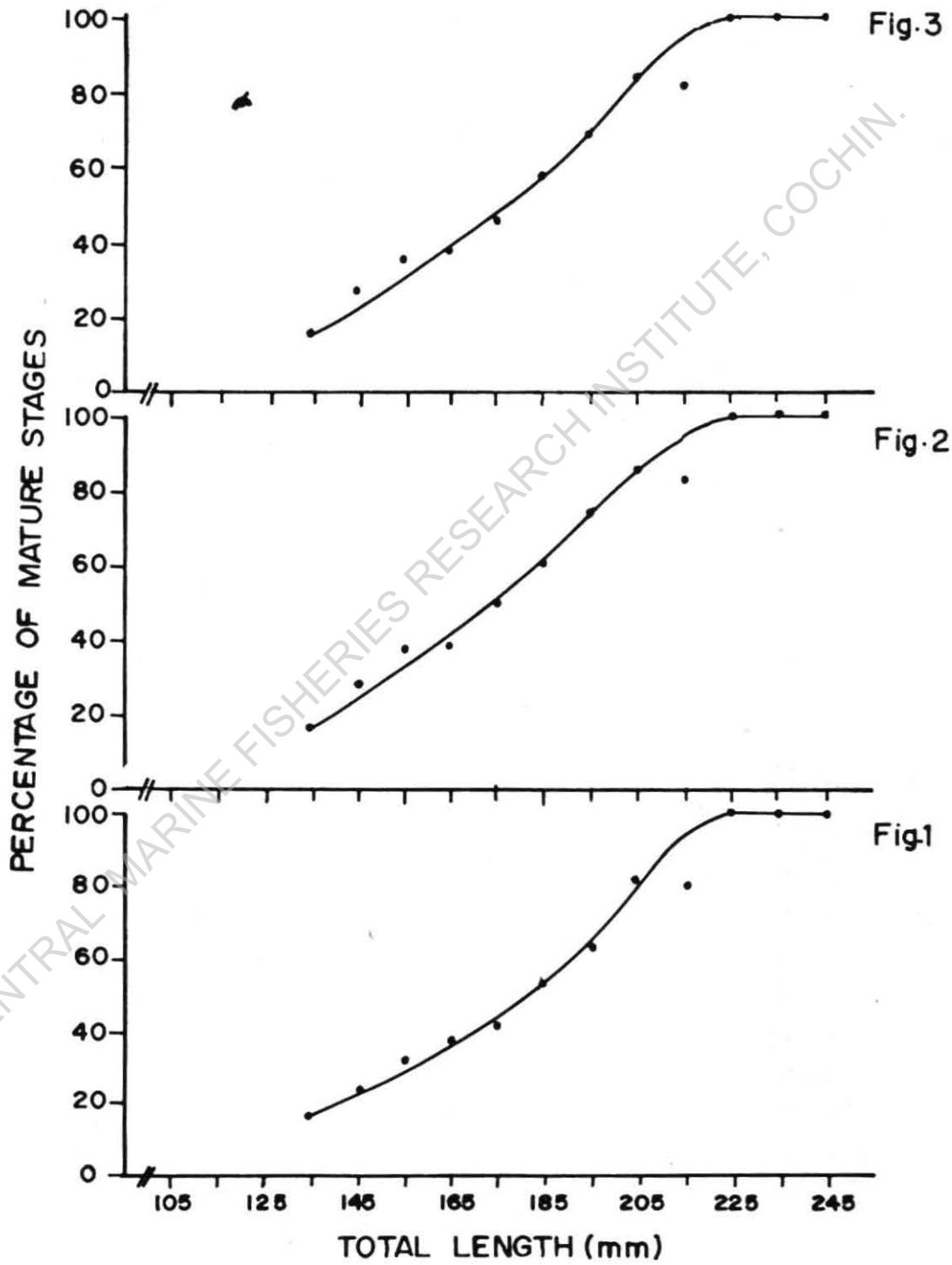
PLATE XII.

Fig.1. Percentage of mature female *S. sihama* in different size groups during July 1984-February 1985.

Fig.2. Percentage of mature female *S. sihama* in different size groups during July 1985-February 1986.

Fig.3. Mean percentage of mature female *S. sihama* of the periods July 1984-February 1985 and July 1985-February 1986, in different size groups.

PLATE XII



group, about 48% were in mature group and in the next size group, 160-169 mm, 61% were found to be mature. Partially spent males were recorded for the first time in this size group. From this size onwards, the percentage of mature fish gradually increased and all of them were mature in 190-199 mm size group (Plate XIII Fig.3).

Data collected on the condition of gonads of both sexes during the period July 1985 to February 1986 gave similar results as above. Females (Table - 17) were found to be in the immature stage upto 129 mm. In the 170--179 mm size group, 33.33% were in stage II and 50% in mature group. In the next group, namely 180-189 mm, the percentage of mature fish has increased to 60%. All fishes were in mature condition in the 220-229 mm size group (Plate XII Fig. 2).

Males measuring upto 129 mm were immature (Table - 19). About 60% of the fish were found mature in the 160-169 mm size group and the percentage of mature fish increased in the following size groups. Partially spent males were found for the first time in 170-179 mm size group. All fish were in mature condition in the 190-199 mm size group (Plate XIII, Fig. 2).

Pooled data for the percentage occurrence of stages III and IV (Mature group) for each year and the average for two years has been calculated (Tables - 20 and 21). It may be seen from Table - 20 that in the 130-139 mm size group, 16.57% and in the 140-149 mm size group, 25.95% of the fish were mature. In the following size group, 150-159 mm, 34.05% were mature and in the next size groups, 160-169 mm, 37.83% were mature and in the next size group, 160-169 mm, 37.83% were mature . In the 180-189 mm size group, 56.67 % of the female fish were found mature. The percentage of

PLATE XIII.

Fig.1. Percentage of mature male *S. sihama* in different size groups during July 1984-February 1985.

Fig.2. Percentage of mature male *S. sihama* in different size groups during July 1985-February 1986.

Fig.3. Mean percentage of mature male *S. sihama* of the periods July 1984-February 1985 and July 1985-February 1986, in different size groups.

PLATE XIII

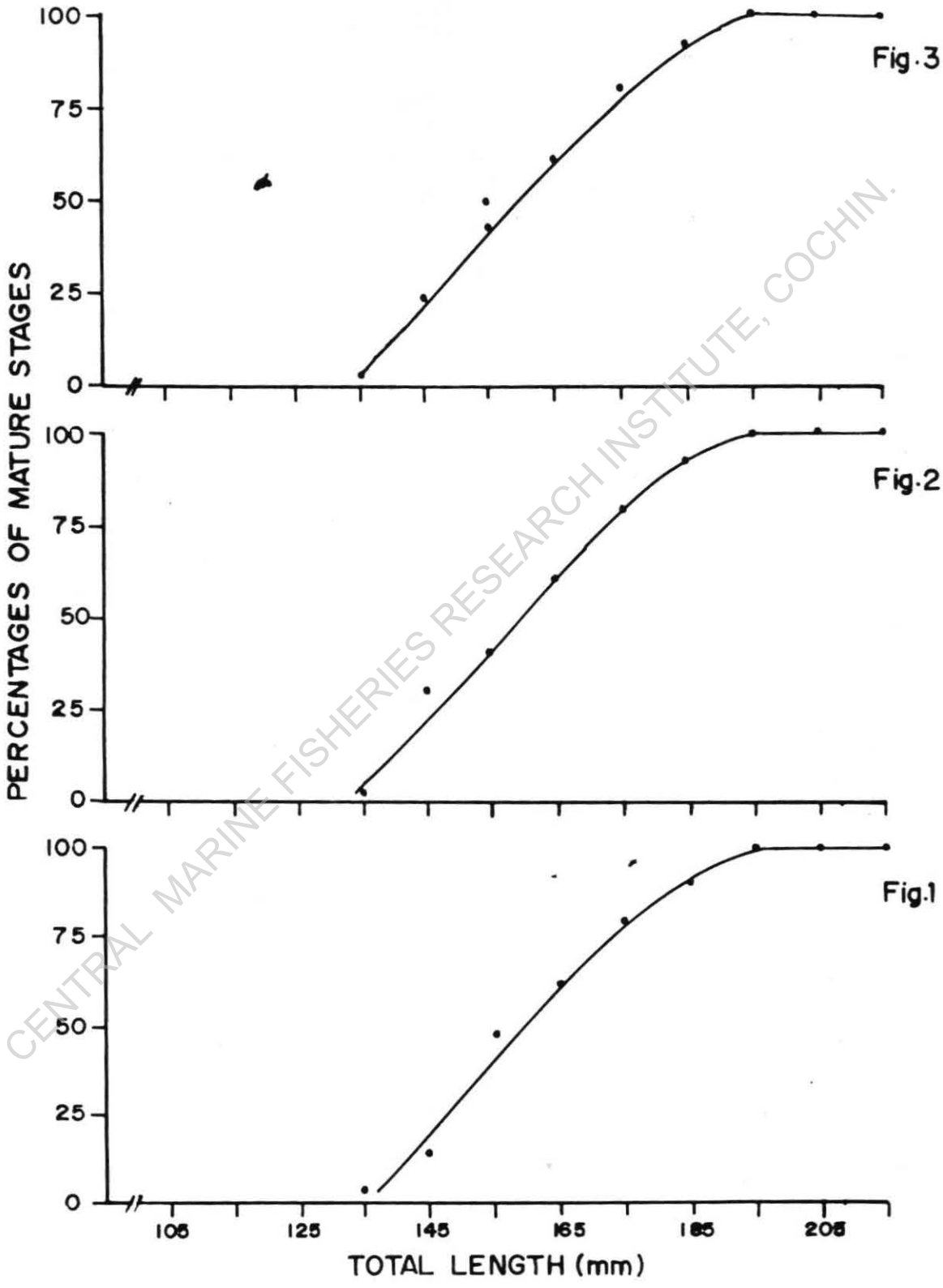


TABLE 20. Percentage occurrence of mature females of *Sillago sihama* in various size groups

Size groups (TL mm)	July, 1984 - February, 1985	July, 1985 - February, 1986	Average
130 - 139	16.00	17.14	16.57
140 - 149	23.33	28.57	25.95
150 - 159	31.43	36.67	34.05
160 - 169	37.33	38.33	37.83
170 - 179	41.06	50.00	45.53
180 - 189	53.33	60.00	56.67
190 - 199	63.33	75.00	69.17
200 - 209	82.35	85.71	84.03
210 - 219	80.00	83.33	87.67
220 - 229	100.00	100.00	100.00
230 - 239	100.00	100.00	100.00
240 - 249	100.00	100.00	100.00

TABLE 21. Percentage occurrence of mature males of *Sillago sihama* in various size groups

Size Groups (TL mm)	July, 1984 - February, 1985	July, 1985 - February, 1986	Average
130 - 139	3.33	3.33	3.00
140 - 149	14.28	28.89	21.59
150 - 159	47.50	40.00	43.75
160 - 169	61.06	60.42	60.74
170 - 179	80.00	80.00	80.00
180 - 189	90.00	93.34	91.67
190 - 199	100.00	100.00	100.00
200 - 209	100.00	100.00	100.00
210 - 219	100.00	100.00	100.00

mature fish increased steadly in the higher size groups and all were mature in the 220-229 mm size group.

It may be seen from Table - 21 that in males in the 130-139 mm size group, 3.33% and in the 140-149 mm size group, 21.59% of the fish were mature. In the 160 - 169 mm size group, as many as 60.74% of the males were found mature and thereafter the percentage of mature fish increased steadily in the higher size groups. All males were mature in the 190-199 mm size group.

The results of the present study show a great similarity in both sexes over two successive years of the study and hence the average results may be indicative of the general trend (Plate XII Fig. 1). For both the years, it may be seen that over 40% of the females were found mature in the 170-179 mm size group and the percentage was found to increase rapidly in the subsequent size groups, which indicates that over 50% of the females are mature at about 179 mm total length. Although from the above data, it is clear that a few fish were found to be mature at 130-139 mm, majority of the female fish attain maturity at about 179 mm total length.

In both the years, over 40% of the males were found to be in the mature condition in the 150 - 159 mm size group and in the following size groups, the percentage of mature specimens increased very rapidly, indicating that over 50% of the males are mature at about 159 mm total length. As in the case of females, few males were found in mature group in the 130-139 mm size group, but majority of male fish attain maturity at about 159 mm total length (Plate XIII, Fig. 1).

Fecundity

For fecundity estimation all ova measuring in diameter between 0.23 and 0.52 mm were counted. They are in maturing and mature stages. It is assumed that both these groups of ova would become ripe and be spawned in the same spawning season. The ripe ova were avoided for fecundity estimation because some of them would be released as soon as they are formed.

Fecundity varied from 6956 to 48,373 in individuals of total length from 150 to 210 mm (Table - 22). The mean fecundity of 17 specimens was 25,979.

Fecundity, in general showed an increasing trend in smaller fish and a decreasing tendency above 204 mm. Total length-fecundity plots showed a curvilinear relationship and plots of body weight and ovary weight versus fecundity appeared linearly related. Fecundity, Total length, body weight and ovary weight were transformed to logarithms (base 10) and by least square method the following equations were obtained:

$$\text{Log } F = -8.1812 + 5.5458 \text{ Log } L; \; r = 0.90$$

where F = fecundity and L = Total length.

$$\text{Log } F = 1.4169 + 1.7418 \text{ Log } W; \; r = 0.89$$

where F = fecundity and W = Body weight

$$\text{Log } F = 0.8107 + 1.1793 \text{ Log } w; \; r = 0.95$$

where F = fecundity and w = ovary weight.

Correlation coefficient (r) was significant ($P < 0.01$) for all three relationships. Scatter diagrams and fitted lines are shown in Plate XIV, Figs. 1-3.

TABLE 22. Variation of fecundity with total length, body weight and ovary weight of *S. sihama*

S.No.	Total (L mm)	Body weight (W g)	Ovary weight (W mg)	Observed Fecundity	Estimated fecundity		
					$F = aL^b$	$F = aW^b$	$F = aw^b$
1	150	28.2	492	6956	7709	8768	9670
2	151	23.8	143	7931	8000	6527	6319
3	160	35.0	500	9000	11020	12770	9884
4	164	37.0	530	11000	12620	14070	10550
5	166	38.5	520	10075	13520	15090	10320
6	170	40.0	850	15000	15430	16120	18420
7	174	43.5	1265	27279	17550	18650	29440
8	175	40.6	1201	30000	18110	16540	27700
9	184	51.8	1471	41296	23930	25280	35180
10	186	49.0	922	22556	25410	22950	20280
11	191	50.0	970	18201	29430	23780	21530
12	198	59.0	1321	45565	35960	31740	30950
13	202	61.0	1400	35245	40190	33610	33180
14	204	74.0	1600	48373	42400	47070	38880
15	206	73.5	1700	47000	44790	47630	41720
16	208	75,5	1800	45725	47260	48730	44640
17	210	79.1	2270	34360	49800	52860	58660

PLATE XIV.

Fig.1. Fecundity-Length relationship

Fig.2. Fecundity-Body weight relationship

Fig.3. Fecundity-Ovary weight relationship

r-Regression coefficient; n-Number of specimens.

PLATE XIV

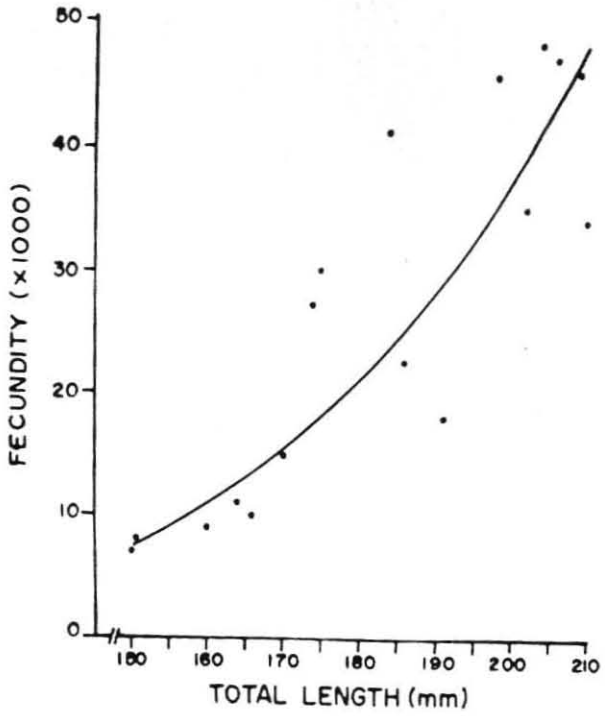


Fig.1

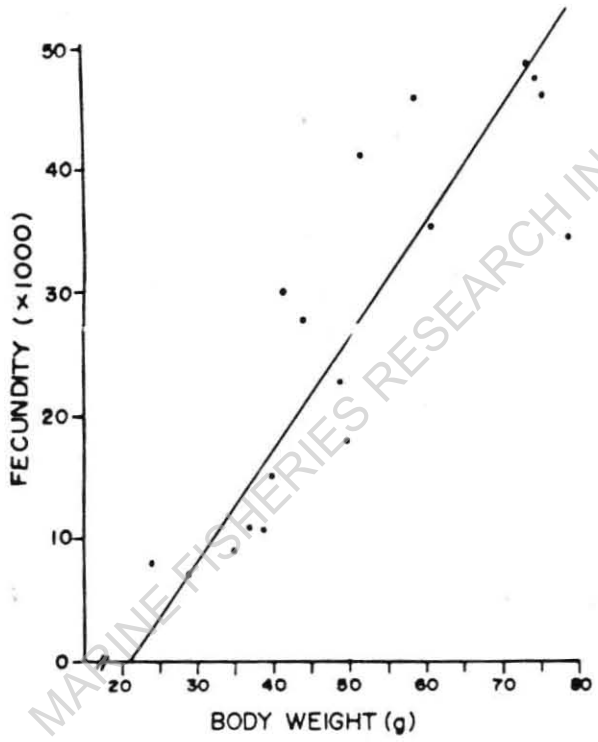


Fig.2

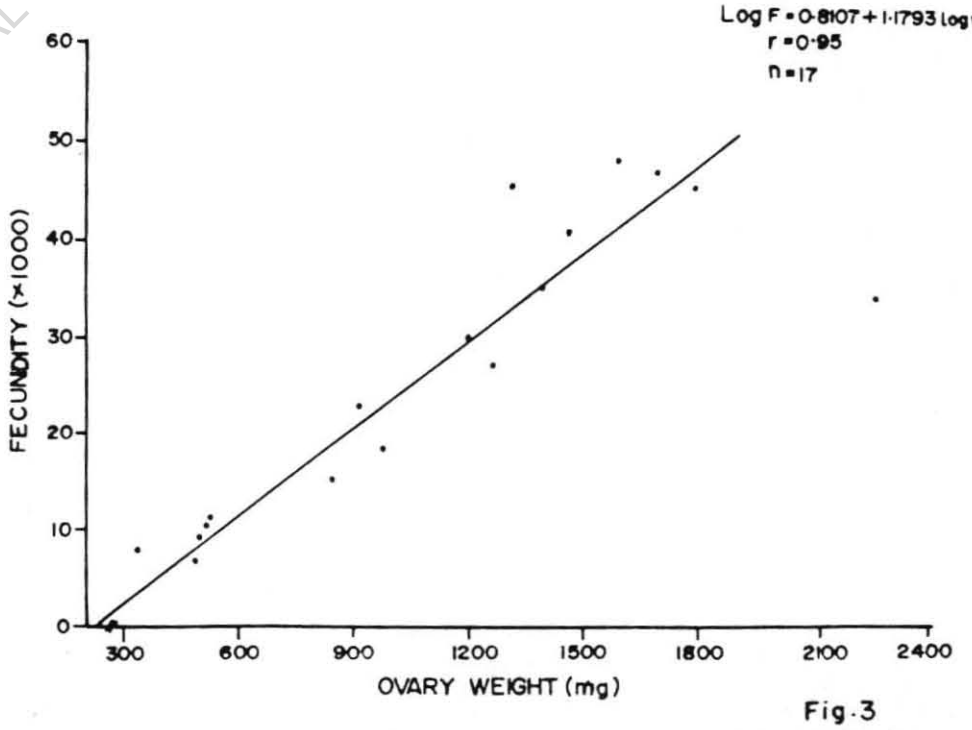


Fig.3

Sex-ratio

Preliminary observations of sex-ratio data of *S. sihama* indicated that there was no noticeable difference in sex composition of samples collected from shrimp trawlers and shore seines. So in all the subsequent samplings the data collected from both the gears were pooled. Since samples were collected from the stake net (*Kalamkattivalai*) only during few months, that data was not incorporated in this account.

Out of 1184 fishes of size ranging from 100-249 mm in total length examined during April 1984 - March 1985, 607 were females and 577 males (Table 23). This gives female: male sex-ratio 1.05:1, which is not significantly different from the expected 1:1 ratio ($\chi^2 = 0.76$, $P > 0.05$). During most of the months, female fish slightly outnumbered males in the commercial catches, but in no month the sex-ratio was statistically different from 1:1 ratio.

Table 25 shows the sex-ratio at 10 mm intervals of total length observed during April 1984 to March 1985. It can be seen that male proportion was greater till 160-169 mm size group. From 170-179 mm size group onwards female fish dominated over male, and in all the size groups above 179 mm, the chi-square value was statistically significant.

The data collected during the succeeding year (April 1985 to March 1986) show similar trend in sex-ratio. Out of 1240 fishes of size ranging from 100 to 249 mm in total length examined during this period, 647 were females and 593 males (Table 24), giving female:male ratio 1.09:1, which is not significantly different from the expected 1:1 ratio ($\chi^2 = 2.35$, $P > 0.05$). As

TABLE 23. Month-wise sex ratio of *Sillago sihama*

(April 1984 - March 1985)

Months	No. of fish examined	Female	Male	Proportion of female	Chi-square
April	120	70	50	1.40	3.33
May	112	48	64	0.75	2.29
June	98	56	42	1.33	2.00
July	80	44	36	1.22	0.80
August	64	35	29	1.21	0.56
September	92	48	44	1.09	0.17
October	72	40	32	1.25	0.89
November	104	59	45	1.61	1.89
December	114	54	60	0.90	0.32
January	84	45	39	1.15	0.43
February	104	48	56	0.86	0.62
March	140	60	80	0.75	2.86

TABLE 24. Month-wise sex-ratio of *Sillago sihama*

(April 1985 - March 1986)

Months	No. of fish examined	Female	Male	Proportion of female	Chi-square
April	138	70	68	1.03	0.03
May	124	60	64	0.94	0.13
June	84	50	34	1.47	3.05
July	98	50	48	1.04	0.04
August	80	42	38	1.11	0.20
September	100	54	46	1.17	0.64
October	114	64	50	1.28	1.72
November	96	56	40	1.40	2.67
December	84	42	42	1.00	0.00
January	80	44	36	1.22	0.80
February	102	50	52	0.96	0.04
March	140	65	75	0.87	0.71

TABLE 25. Sex-ratio of *Sillago sihama* in various size groups

(April 1984 - March 1985)

Size groups (TL mm)	No. of fish examined	Female	Male	Proportion of female	Chi-square
100 - 109	6	2	4	0.50	0.67
110 - 119	22	12	10	1.20	0.18
120 - 129	40	10	30	0.33	10.00**
130 - 139	80	36	44	0.82	0.80
140 - 149	116	56	60	0.93	0.14
150 - 159	149	58	91	0.64	7.31 **
160 - 169	227	102	125	0.82	2.33
170 - 179	193	117	76	1.54	8.70**
180 - 189	166	100	66	1.52	6.96**
190 - 199	122	88	34	2.59	23.90**
200 - 209	38	36	2	18.00	30.42**
210 - 219	10	10	0	-	10.00**
220 - 229	7	7	0	-	7.00**
230 - 239	4	4	0	-	4.00*
240 - 249	4	4	0	-	4.00*

* $P < 0.05$ ** $P < 0.01$

TABLE 26. Sex-ratio of *Sillago sihama* in various size groups

(April 1985 - March 1986)

Size groups (TL mm)	No. of fish examined	Female	Male	Proportion of female	Chi-aquare
100 - 109	10	4	6	0.67	0.40*
110 - 119	27	13	14	0.93	0.04
120 - 129	46	15	31	0.48	5.57*
130 - 139	87	42	45	0.93	0.10
140 - 149	116	53	63	0.84	0.86
150 - 159	181	81	100	0.81	1.99
160 - 169	235	120	115	1.04	0.11
170 - 179	190	120	70	1.71	13.16**
180 - 189	150	100	80	2.00	16.67**
190 - 199	78	70	8	8.75	49.28**
200 - 209	69	65	4	16.25	53.93**
210 - 219	21	21	0	-	21.00**
220 - 229	12	12	0	-	12.00*
230 - 239	10	10	0	-	10.00**
240 - 249	8	8	0	-	8.00**

* $P < 0.05$ ** $P < 0.01$

in the previous year, during most of the months, female fish slightly outnumbered males in catches, but sex-ratio was not significantly different from the expected 1:1 ratio in any of the month.

Table 26 shows the sex-ratio at 10 mm intervals of total length observed during April 1985 to March 1086. Male fish dominated till 150-159 mm size group. From 170-179 mm onwards female:male sex-ratio was statistically different (in favour of females) from 1:1 ratio ($P < 0.01$).

DISCUSSION

The morphology of the female and male redproductive systems in *Sillago sihama* were typically teleostean, with a pair of gonads lying ventral to the swimbladder in the body cavity, united posteriorly through a common duct which opened to the exterior through a cloaca.

Different criteria, such as the size of the gonad in relation to the body cavity, ova diameter, fat content and gonadosomatic index were used by various workers in order to classify the maturity condition in fish. Eight maturity stages were described in herring (Hjort, 1910), which were further modified by the International Council for the Exploration of the Sea (ICES) in 1962. However, this classification was developed primarily for the temperate water fishes which have a definite spawning season within which the stages of maturity are fairly uniform throughout the population at any one time. Majority of the tropical fishes have prolonged breeding season. In these species, almost all the maturity stages may be available throughout the year (Clark, 1934; June, 1953; Yuen, 1955; Yuen and June, 1957; James and Baragi, 1980), and hence any classification of maturity based on the models of temperatre forms will not give a correct picture of the breeding season (Qasim, 1973). In such fishes, the maturity scale should be based more on the modal positions of different batches of ova (James and Baragi, 1980).

Qasim (1973) suggested that in tropical and subtropical forms, the maturity classification should be limited to five stages, namely immature, virgins, maturing virgins (or recovering spents), ripening, ripe and spent. He has further remarked that in continuous breeders, a fully ripe stage and

completely spent stage shall not be included, as according to him "the inclusion of these stages in the classification would be superfluous and misleading". Difficulty in collecting running ripe and spent stages in several fishes have been reported (Clark, 1934; Radhakrishnan, 1957; Hopson, 1969; Knaggs and Parrish, 1973; Leary *et al.*, 1975; Hunter and Goldberg, 1980; Morse, 1980, 1981; Goldberg, 1981; Davis, 1982). In *Scomber japonicus*, Knaggs and Prish (1973) could collect but only 5 spent fishes in the entire period of their study spanning almost a decade. According to them, "since eggs mature in successive batches, a spent condition would not be found in a fish until all spawnings are completed". Working with the reproductive biology of liparid fishes, Stein (1980) observed very few spent specimens of *Osteodiscus cascadidae* and *Acantholiparis opercularii* and no spent specimens of *Paraliparis latifrons* in Oregon waters. No female fish of *Genyonemus lineatus*, *Seriphus politus* and *Cheilotrema saturnum* in post spawning condition (partially or fully spent stage) could be collected by Goldberg (1976, 1981). Similarly, no spent stage of *Scomber scombrus* and *Paralichthys dentatus* were described (Morse, 1980, 1981). Radhakrishnan (1957) has stated that spent fishes were not available during his study on *Sillago sihama*.

In the present work, the maturity of female fish has been classified into five stages, immature, maturing, mature, ripe and partially spent based on the general appearance of ovaries and ova diameter measurement and that of male fish into five stages, immature, maturing, mature, oozing and partially spent based on the size, shape and colour of testes. Female in ripe running condition was not found. The duration of this stage can be so brief that on may fail to detect it.

There are variations in the maximum size of the ripe egg of *Sillago sihama* as reported by different workers: 0.517 mm (Radhakrishnan, 1957); 0.8 mm (Palekar and Bal, 1961); 0.768 mm (James *et al.*, 1976); 0.782 mm (Kumai and Nakamura, 1977) 0.783 mm (present study). Radhakrishnan (1957) has collected the specimens from the same region as in the present work. But he has remarked that the size of the fully ripe ovum could be more than what he has recorded.

Descriptions of reproductive cycles of teleosts are normally based on examination of ovaries alone. Male fish are generally avoided because of the difficulties in assigning maturity stages. However, in the present work, reproductive cycle of male fish too, was studied, with a view to understanding whether it is adequately synchronising with that of the female.

Ova diameter study is a useful tool to know the spawning period and spawning frequency in fish. Based on the measurement of ova in the ripe ovary, evidence of the duration of spawning in a fish, ie., whether the spawning period is short and definite or long and indefinite, could be obtained (Hickling and Rutenberg, 1936). These workers and deJong (1940) have classified spawning habits of teleosts into four types:

(1) A short spawning, once in a season. The mature ovaries show immature and mature ova distinctly separated from each other.

(2) Spawning takes place once, but over a long period. The size range of mature ova be approximately half of the total range in size of ova.

(3) Spawns twice in a season. Ovaries with mature ova and another group of ova which has undergone about half the maturation process.

(4) Spawns intermittently over a long period. Ovaries have successive batches of ova which are not sharply differentiated.

In a number of Indian marine teleosts this method has been applied to determine the spawning period (Prabhu, 1956; Luther, 1963; Venkatasubba Rao, 1963; Antony Raja, 1964; Raju, 1964; James, 1967; Venkataraman, 1970; Devaraj, 1977).

The ova diameter frequency polygon of *S. sihama* showed a decreasing trend in the percentage of immature ova with respect to the maturing group as maturation process advanced, and reached the lowest value at the beginning of spawning (Ripe stage). Thus, at a specific stage of maturation, immature eggs stopped growing and no additional immature eggs joined the advanced group. In the ripe ovary, four batches of ova were demarcated. The most advanced batch of ova was represented by a mode at 0.68 mm. These ova were transparent with oil globules and constituted the first batch that would be spawned first. Two more groups of ova with modes at 0.38 mm and 0.23 mm followed this group. The former group contained both maturing and mature stages of ova, which were not sharply differentiated from each other, while the latter group contained only maturing ova. The rest of the ova belonged to the immature group. The succession of maturing groups of ova indicates that each individual fish spawns more than once. Such a condition is supported by the presence of partially spent stage fish during many months. The partially spent condition could be confirmed by the presence of residual eggs, which were almost transparent with oil globules. They were mostly shrunken, distorted in shape and thus destined to degenerate. The ovary in this stage, resembled more or less the maturing ovary. 'Reversion' of ovary from the partially spent condition to the earlier stages has been well documented (James and Baragi,1980,.

The sharp increase in the proportion of maturing eggs in the Partially spent ovary compared to the ripe one, as seen in the present study, indicates that different batches of eggs will be passing from one stage to the other. Thus, the pattern of ova development in *S. sihama* would indicate a prolonged breeding season in the species, and the fish may fall into the fourth category of the classification of spawning types mentioned above. However, it is not possible to define too rigidly the spawning type in this fish based on oocyte distribution. For example, according to the above mentioned classification of spawning types, in the fourth type there would be no separation at all among the batches of ova. In *S. sihama*, the most advanced clutch of ova was clearly separated from the mature and maturing groups of ova.

Based on ova diameter studies, James *et al.*, (1976) have suggested a prolonged breeding season in *S. sihama* in Nethravathy and Gangolly estuaries, while Palekar and Bal (1961) found a comparatively brief spawning period for the same species in Kali river estuary. The present results are in conformity with those of James *et al.* (1976).

Ovary has also been used as an indicator of the frequency of spawning in fishes (Clark, 1934; Hickling and Rutenberg, 1936; June, 1953; MacGregor, 1957; Luther, 1973; Devaraj, 1977; James and Baragi, 1980). Clark (1934) pointed out that, if only one batch is spawned, the ratio between the number of eggs in the maturing group and the number of eggs in the mature group should remain constant and, on the other hand, if more than one batch is spawned, the ratio gradually decreases. Based on this principle, she proved that individual California sardine spawns an average of three batches. For inferring multiple spawning, she provided four lines of evidence, namely, multiplicity of modes in the ova diameter frequency curves, a high degree of

correlation between the growth of successive groups eggs, occasional presence in the ovary of a few rupe, unspawned eggs and the decreased in the ratio of the number of eggs in the maturing groups and the mature group as the breeding season advances.

Luther (1973) based on ova diameter studies, found that three batches of ova are shed by *Rastrelliger kanagurata*. from Andaman islands. Dhulkhed (1967) expressed the view that eggs of the more advanced mode in oil sardine show differential ripening and consequently are released in three to four batches during the season. Thomson (1957) reported spawning in three batches in *Sillago schomburgskii*, while Cleland (1947) postulates at least two spawnings per year judging the trimodality of ova diameter frequency diagram in *Sillago ciliata*. Morse (1980, 1981) found that the ratio of egg number in the most advanced mode and all the other yolked ova averaged 16-17 percent in *Scomber scombrus* and *Paralichthys dentatus*, and indicated that approximately 6 batches of eggs would be spawned per individual each year.

In the present work, the ratio of the ripe ova and all other ova $\geq$ 0.23 mm (yolked) averaged 37 percent and this may indicate that approximately 3 batches of eggs would be spawned by an individual female during the spawning period. However, this estimate of batch size could be inaccurate. As reported by Macer (1974), such estimates assume that egg batch size remains constant throughout the spawning season and that all eggs in the advanced mode are shed at one time. It was not possible to confirm either of these conditions in the present study. *Sillago japonica* spawned almost every day during the spawning season extending from June to October, in capacity (Kashiwagi *et al.*, 1984). Kumai and Nakamura (1977) noted that a single female *Sillago sihama* of 201 mm (Fork length) in capacity.

spawned 65 times during a 108-day period, spawning every day or every other day. This observation seems to suggest that the most advanced mode of ova may be released in several batches, thus making if difficult to accurately estimate the batch size in this species.

Prolonged spawning periods are characteristic of tropical and subtropical species of fishes which exist in the lower latitudes, while comparatively short spawning seasons characterise the higher latitude temperate species (Qasim, 1956; Nikolsky, 1963; Munro *et al.*, 1973). A number of authors have given as a reason for long tropical spawning seasons the fact that the lower the latitude, the longer the season when temperature and food conditions favour the survival of juveniles (Qasim, 1956; Harden-Jones, 1968). In higher latitudes, the existence of favourable temperature threshold has been considered of much importance for favouring reproduction (de Valaming, 1972 a,b; Qasim, 1973). On the other hand, in tropical waters, where variations in sea temperature and food supply are not so well-marked, these two factors do not seem to act as trigger stimuli for breeding (Qasim, 1973). However, annual changes in temperature and salinity may affect the coastal fishes to some extent in their breeding season. Radhakrishnan (1957) observed that *Sillago sihama* appeared to breed in colder season in Palk bay and Gulf of mannar. Antony Raja (1972) has related the poor spawning in oil sardine to poor rainfall during the monsoon period.

The percentage occurrence of different maturity stages of female and male *S. sihama* during months showed fair degree of similarity between sexes and years. March to June period appears to be the 'resting' phase, when only a small proportion of the fish is engaged in spawning. In March

no female fish in ripe stage could be collected while male fish in 'oozing' condition was encountered. This may be attributed to the fact that spermatogenic activity is longer than ovarian maturity in fishes (Brusle, 1981). In Berre, ovarian ripeness of *Mugil captio* decreased and stopped in November but mature males were still observed in February and March (Ezzat, 1965). Thierberger-Abraham (1967) found that male *Mugil cephalus* have a longer spawning peak than females, so fertilization is assured; it is evident, that for propagation of the species, male and female reproductive activity must synchronise, but it is of great importance that male maturation was closely parallel to that of females during the time of spawning (Brusle, 1981).

From July to February period, there was occurrence of fish with imminent spawning condition (stage V), and partially spent stage was also persent during this period. Hence it may be inferred that *Sillago sihama* has a 8 month-long breeding period in the present study area. The earlier work (Radhakrishnan, 1957) in the same region, showed that spawning in *Sillago* takes place from August to February with a peak in October. James *et al.* (1976) reported that the breeding season *S. sihama* in Nethravathy and Gangolli estuaries extends from August to April. However Palekar and Bal (1961) observed a short duration of spawning activity in the same species, from August to October, in Kali river estuary. In Japan, the spawning season of *S.sihama* is reported to extend from June to September (Lee and Hirano, 1985). Protracted spawning season has been recorded in other sillaginids also. In West Australian waters, the spawning period of *Sillago Schomburgskii* occurs between October and February (Thomson, 1957). In Hooghly estuary, *Sillaginopsis panijus* has been reported to spawn twice a year during

the months November to February and August to September (Krishnayya, 1963). In *Sillago ciliata* (Cleland, 1947) also at least two spawnings per year has been found.

While studying the seed resources at Mandapam, seed of *Sillago sihama* measuring 12-80 mm were collected in large numbers during August and November in 1978 and January, February, May, June and July in 1979 on the Palk bay side (James, 1984). That the seed is available in several months is suggestive of a prolonged spawning season of the species in this region. However, it was not possible to study the exact spawning grounds in the Palk bay or Gulf of mannar. Chaudhuri (1923) commented that *S. sihama* breeds either in the sea or in the mouth of Chilka lake, in which this species has been found to be a permanent resident. A similar observation was made by Cleland (1947), who presumes that *Sillago ciliata* spawns either in the mouths of rivers or more probably in the open sea. In the present work, both female and male fishes in stages IV and V were hardly seen in the shore seine catches, while even maturing stages were rare in the samples collected from 'kalamakattivalai', both the gears being operated in inshore waters. From this observation, it may be inferred that *S. sihama* is likely to breed in the open sea in Palk bay and Gulf of mannar. Palekar and Bal (1961) have reported that marine specimens of *S. sihama*, obtained from inshore catches, when examined, were generally found to be in the early stages of maturation only.

Protracted spawning season cannot always be aquated with multipile spawns of each individual female fish. On the other hand, it could simply reflect a lack of population synchrony in terms of gonadal development (de Vlaming, 1983). Thus, in a fish species in which a prolonged breeding

season is suspected, should be subjected to very extensive sampling to know whether all the individual fishes are maturing simultaneously. De Vlaming (1983) has commented that the terms 'multiple spawner' and 'fractional spawner' should not be equated. The term 'multiple spawner' is generally applied to a species in which the female spawns more than once in a spawning season. The term 'fractional spawner' has been used to refer to a species which spawn a part of ovulated clutch or which mature, ovulate and spawn a part of the post vitellogenic clutch at intervals over a relatively short period. Since egg batch estimate size of *S. sihama* could not accurately be determined in the present study, it is rather difficult to say to which category the species actually belongs, but since more than one spawning per season is confirmed the fish may be said to be a 'multiple spawner'.

The reproductive cycle is reflected by pronounced variations in gonadal size. When assessing gonadal activity, animals of different sizes are frequently sampled and it is generally assumed that gonadal weight depends on animal size and stage of gonadal development (de Vlaming *et al.*, 1982). Nikolsky (1963) states that, "the effects of fish size on gonadal weight are eliminated by expressing gonadal weight as a percentage of body weight". Thus, in work with fishes, the gonadosomatic-index (GSI) is widely used as an index of gonadal activity and as an index for spawning preparedness.

The data relating to changes in GSI of *S. sihama* show that the mean monthly GSI reflects in broad terms the gonadal activity of the female and male fishes in the population. There was considerable similarity in the GSI variation over the two successive years between sexes. GSI values were high as well as variable during July to February period (spawning season), with maximum value in November.

GSI is not always the best way of expressing a gonadal index. It is possible to mask information in a species where gonadal activity is not completely synchronised. de Vlaming *et al.* (1982) comment that use of GSI is appropriate only when it is confirmed that gonad weight has a positive correlation with body weight of the fish. They are of the opinion that expressing the ovarian weight as an exponential function of some measure of body size may provide a more appropriate gonadal index.

In the present work, the female fish was found to mature for the first time at about 179 mm TL and male fish at about 159 mm TL. The smallest size of mature fish (both sexes) measured 139 mm TL. Radhakrishnan (1957) determined the age of *Sillago sihama* by otolith study and reported that fishes measuring 160-200 mm TL were 2 years old. Based on this observation. it may be assumed that both sexes in the present study attain first maturity in the second year. Kakuda (1970) noted that out of 98 one year old specimens of *S. sihama* collected from Inland sea of Japan, only 8 females were found to have ripe ovarian eggs and that all the fish older than two years of age were provided with ripe eggs. Two other species of *Sillago*, *S. schomburgskii* (Thomson, 1957) and *S. ciliata* (Cleland, 1947) are also reported to attain maturity when they are 2 years old. However, Radhakrishnan observed that *S. sihama* matures for the first time at 130 mm (1 year old). James (1976) working with the same species at Nethravathy and Gangolli estuaries, found that the males and females mature at 151 mm and 191 mm TL, respectively. At Kali river estuary, the female *S. sihama* attain first maturity at 235 mm TL (Palekar and Bal, 1961) .

The general observation that males mature at a smaller size compared to the females, could be reflective of the longer life span in the latter than that of the former. Gandolfi and Orsini (1970) (as quoted by Brusle, 1981) have observed that in Venetian lagoon, most males of *Mugil saliens* reach first sexual maturity in the second year, while the females ripen in the third year. Most of the males of *Macquaria novemaculeata* were sexually mature by age 3+, and females 5+ or 6+ years (Harris, 1986). Male precocity has also been shown in *Mugil chelo* (Erman, 1961; Hickling, 1970; Farrugio and Quignard, 1973).

The size at which a species becomes mature is a rather constant proportion of the final length or asymptotic length (Holt, 1962). Cushing (1968) observed that bigger the fish, bigger it is at first maturity. The ratio of the mean length at first maturity (L_m) to the asymptotic length (L_x), referred to as 'reproductive load' could be useful in reproductive studies (Beverton and Holt, 1959).

Since the success or failure of a fish species largely depends on its spawning potential, the knowledge of fecundity becomes extremely important from the view point of successful management and exploitation of its fishery. In broad terms, fecundity may be defined as the number of eggs produced by an individual during its life-time (Lowe-McConnell, 1975). Bagenal (1978) finds this definition unsatisfactory because of the difficulties involved in determining such a figure, but nonethless, for the purposes of examining reproductive strategies in fish, it would be the most meaningful. However, most authors define fecundity in more practical terms as the number of ripening eggs in the ovary just before spawning and even here problems arise when studying multiple spawners (Macer, 1974; de Silva, 1973) and total spawners that may spawn more

than once a season. Macer (1974) remarks that in serial spawners, fecundity would appear to be more flexible, since the process of asynchronous development and oocyte resorption make possible to control egg numbers during the current spawning season. In his study with *Trachurus trachurus*, he has selected all the yolked ova barring the ripe ones for fecundity estimation. In the present study also the same method was adopted. The fully ripe ova have to be avoided from consideration, since soon after their formation, a portion could have been spawned. In *S. sihama*, significant yolk accumulation was seen in ova larger than 0.23 mm and all ova $\geq$ 0.23 mm, except the ripe ones ($>$ 0.52 mm) were counted for fecundity estimation. This may be considered as the maximum potential egg production. A similar method was adopted in *Pleuronectes platessa* (Bowering, 1978), *Scomber scombrus* and *Paralichthys dentatus* (Morse, 1980, 1981). James *et al.* (1976) have defined the fecundity of *S. sihama* from Nethravathy and Gangolly estuaries as the total number of largest group of ova present in the ovary at the time of capture of fish. Their figure could apparently be an underestimate since the ova once ripened would soon be ovulated and spawned.

Fecundity estimates of *Sillago sihama* reported by different workers show variations. Radhakrishnan (1957) stated that a fully mature ovary of the fish contained 14,000 eggs, but has not indicated the size of the ova or other criteria for fecundity estimation. Fecundity of *S. sihama* form Kali river estuary varied from 16,682 to 166,130 in individuals ranging in total length from 184 mm to 340 mm (Palekar and Bal, 1961). They seem to have counted all the ova in the ovary, though no mention has been made about the criteria for fecundity estimation. Fecundity of this species from Nethravathy and estimation. Fecundity of this species from Nethravathy and Gangolly estuaries

ranged from 11,304 to 1,00,593 in individuals measuring in total length from 207 to 317 mm (James *et al.*, 1976). In the present study, the fecundity (all ova in the range of 0.23-0.52 mm) varied between 6956 and 48,373 in individuals of total length ranging between 150 mm and 210 mm. Kumai and Nakamura (1977) have reported that a female specimen of *Sillago sihama* measuring 201 mm fork length has spawned a total of 1,800,750 eggs during a 108-day period in captivity.

The fecundity is affected by a number of factors and according to Simpson (1951) these factors might be:

(a) the condition of the fish when the germinal epithelium is laid down during the first year of life, and

(b) the condition of the fish either when the eggs to be laid each year are separated from the mass of developing ova, or when the new primary oocytes are being formed each year. The condition of the fish at these critical times is expected to be closely associated with the food supply and the temperature of the environment.

Simpson (1951) states, "all observations on the fecundity of fish have shown that for fish of any species the fecundity increases with the size of the fish". Lack (1954) and Bagenal (1957) have also confirmed that fecundity is associated more with the size than age. The correlation coefficients for the relationships of fecundity with length, body weight and ovary weight showed, in a number of cases, that ovary weight was most closely associated with the variation in fecundity. But body weight and ovary weight are subjected to considerable seasonal changes and thus length appears to be the most reliable and convenient measure for general prediction of fecundity. Generally an

exponential or non-linear relationship has been noticed between fecundity and length (Raitt, 1932; Hickling, 1940; Simpson, 1951; Bagenal, 1957; Qasim and Qayyum, 1963; Morse, 1980, 1981), which indicates that fecundity increases more rapidly than the length of the fish. However, a linear relationship between fecundity and length has been observed by certain workers (Lehman, 1953; Jerald and Brown, 1971; Mathur and Ramsey, 1974; Muth and Tarter, 1975). Fecundity bears linear relationship with body weight and ovary weight in most fish species, which signifies that the number of eggs in the ovaries increases in proportion to the weight of the fish and also to that of its gonads (Raitt, 1932, Bagenal, 1957; Pope *et al.*, 1961; Pantulu, 1963). Some workers could not find any relationship of fecundity with body size (James, 1967; Mohan, 1977).

In the present work, the fecundity of *S. sihama* bore a curvilinear relationship with total length, and linear relationship with body weight and ovary weight, with high correlation coefficients in all the three relationship between fecundity and length of *S. sihama* collected from Kali river estuary. Simpson (1951) expressed the relation between fecundity and length by the "cube law", $F = KL^3$. Other investigators, such as Bagenal (1963), Pitt (1964) and May (1967) in studying fecundity of other species, used this relationship and found that, more often than not, the exponent of the equation fell between 3 and 4. However, in the present study, the exponent of this equation was found to be 5.55. In Palekar and Bal's (1961) work with the same species, the value was 4.33. It is not uncommon to find such situations in which fecundity was found to vary well above the cube of length, for example, 4.5 power of the length in Irish sea herring (Farran, 1938), 5.4 power of the length in *Mystus vittatus* (Qasim and Qayyum, 1963), etc. Evidently these relationships singnify a high fertility in these species, including the present experimental fish. It is also probable that

this condition exists in smaller fish. Once these fishes become older, the growth of length becomes quite slow and may practically stop, while at the same time the ovaries continue to grow. This explains the fecundity following a relationship higher than the cubic parabola in these species (Qasim and Qayyum, 1963). Simpson's (1951) conclusion offers further evidence to such a differential growth rate; it says, "it is quite conceivable that an organ such as the ovary, which undergoes such great changes in size during each year, might progressively form a somewhat larger or smaller proportion of the fish as it grows old and larger, and so not give rise to a perfect cube relationship with length".

Sex composition in a fish population might be affected by the following factors (El zarka and El sedfy, 1970):

(i) segregation of the sexes through various periods of the year including segregation resulting from sex differences in age and size at maturity

(ii) gear selectivity in relation to sex differences in morphology and in physiological activity, and

(iii) differences in natural and fishing mortality between the sexes.

The sex which exhibits a faster growth rate will be less affected by predation and this would influence the sex-ratio. Further, that the existence of a size hierarchy in the population will favour the large-sized individuals in both intra and interspecific competition for food and space may also affect the sex-ratio. In otherwords, it appears that survival is a function of length (Qasim, 1966).

In several fish species, the ovarall sex-ratio was in favour of female sex (*Sillago ciliata* : Cleland, 1947; *Hilsa ilisha* : Jones and Menon, 1951; *Hippoglossoides platessoides:* Bagenal, 1957; Mugil saliens: Gandolfi and

Orsini, *Op. Cit; Liza parsia:* Kurup and Samuel, 1983), while in others male fish has preponderence in the population (*Sillago sihama* : Radhakrishnan, 1957; *Tilopia nilotica*: Babiker and Ibrahim, 1979; *paralichthys dentatus* Morse, 1981).

The monthly sex-ratio of *Sillago sihama* over two successive years in the present work, shows a slight preponderence of the female sex during most of the months, though the sex-ratio was statistically not different from 1:1. Further, there was no apparent difference in the sex composition between the samples collected from the shore seines and the shrimp trawl. Gandolfi and Orsini (*Op Cit.*) have observed a higher proportion of female *Mugil saliens* in lake Qarun especially during the breeding season and have attributed this to the greater activity of female sex during the breeding season which is turn increased the chances of being caught. Similarly, Jones and Menon (1951), while working on the hilsa at Hoogly river, found preponderence of females during the breeding season, though the sex ratio was statistically not different from the expected 1:1. In the population of *Sillago ciliata* in New South Wales and Queensland, Cleland (1947) observed that the sex-ratio differed significantly from the expected 50:50 ratio, being found to be 47.5 males to 52.5 females. However, Radhakrishnan's(1957) observation of overall sex-ratio of *S. sihama* from Palk bay and Gulf of mannar (male : female :: 55.6:44.4) is not in agreement with the present results. The observed trend in sex-ratio in the present work seems not to due either to behavioural difference or geat selectivity.

Radhakrishnan (1957) noticed that male formed a higher percentage than the females in the size groups upto 170 mm TL in the population of *S. sihama* Over the two successive years, sex composition in different size groups of the same species in the present study, showed that male fish has a preponderence

over the other sex < 170 mm TL, while female fish dominated the population > 170 mm TL, and there was no single male fish > 209 mm TL. This could be the result of differential growth rate between the sexes and longevity. A similar observation was made in *Paralichthys dentatus* (Morse, 1981).

The dominance of males between 100-169 mm TL and the difference in size at first maturity for either sex appears to maintain a 1:1 sex ratio in the spawning population. It has already been seen that the female and male *S. sihama* matures for the first time at 179 mm and 159 mm TL, respectively. By combining the data given in Tables 25 and 26, the sex ratio of females >180 mm TL to males >160 mm TL would be 1:1.02, which shows a slight preponderence of the male sex. If the sex ratios are adjusted to a 1:1 ratio at the 154-164 mm size group, and also assuming that females sex has a greater growth rate and longevity, the total female to male sex ratio becomes 1.8:1, with a significant dominance of females. Thus, it appears both length at first maturity and differential sex ratios by length combine to produce equal numbers of each sex in the spawning stock.

The foregoing account has shown that *S. sihama* is a high fecund fish with multiple spawning and protracted breeding period. This reproductive strategy might reduce larval crowding and decrease the impact of predators and adverse environmental conditions on egg and larval survival.

CHAPTER V

HISTOLOGY OF GONADAL DEVELOPMENT

A correct picture of maturation and spawning in fish can hardly be accomplished by the arbitrary classification of gonads. Similarly, conventional methods such as the study of the progression of oocytes, only give evidence of an exploratory nature on spawning (Qasim, 1973). Histological and cytological studies give greater details of spermatogenesis and oocyte growth. Two of the great advantages of histological classification are that, firstly the frequency of multiple spawning fish populations can be accurately estimated, and secondly regressing gonads could be distinguished from immature and post-ovulatory ones (Hunter and Macewicz, 1985).

The processes involved in the formation of gametes have been extensively investigated by the use of histological, histochemical and electron microscopical techniques. Some excellent reviews on this subject covering most of the important literature include Raven (1961), Lofts (1968) Dodd (1977), Wallace (1978), Grier (1981), Wallace and Selaman (1981), Billard (1982), deVlaming (1983) and Naghama (1983).

Oocyte development in fish takes place mainly in two phases. During the first phase,oocyte increases in size with some nuclear changes and during the second, yolk deposition takes place. A series of complex morphological and cytochemical changes take place during this process.

In the past few years, voluminous literature on the histological changes occurring in the ovary during its maturation have been published. Some of the earlier works on this subject include Hickling (1935) in *Merluccius merluccius*,

Turner (1938) in *Cymatogaster aggregatus* and a series of papers by Yamamoto,K. (1955a , b; 1956a, b, c, d, e, f; 1957b; 1958a & b) on *Hypomesas japonicus*, *Liopsetta obscura* and *Clupea pallasii*. More recently Davis (1977, 1982) furnished lucid picture of gonadal histology in *Tandanus tandanus* and *Lates calcarifer*. Hunter and Macewicz (1985) studied rates of atresia in *Engraulis mordax* ovaries.

Spermatogenesis in teleost fishes, includes a series of cytological events that begin with the origin and differentiation of primordial germ cells and terminate with the release of the mature spermatozoa into the lumen of the seminiferous lobules. Using standard histological and staining methods and by using light microscopy, several workers have studied gametogenesis in male teleost fish. A few noteworthy studies include spermary in perch (Turner, 1919), sperm development in *Oryzias latipes* (Grier, 1976), cyclic changes in testicular lipids in *Esox lucius* (Lofts and Marshall, 1957), seasonal cycle in the testes of *Fundulus heteroclitus* (Mathews, 1938) and the structure of spermatozoan in *Liza dumerili* (Van Der Hurst and Gross, 1978).

During the past one and half decades, there has been considerable expansion in the knowledge of fish gametogenesis by the application of cytological and electron microscopy, which enable the workers to understand the different aspects of origin and development of the reproductive elements at the ultrastructural level (for review, Nagahama, 1983).

Among the Indian teleosts, majority of studies on histology of reproduction have been carried out in fresh water species, such as *Heteropneustes fossilis*, *Schizothorax* spp., *Clarias batrachus* and *Channa* spp. Follecular atresia, which is a degenerative process by which oocytes in different stages of maturity

are lost from the ovary, has received the attention of some workers, such as Rajalakshmi (1966) in *Gobius giuris*, Samuel and Khanna (1972) in *Channa gachua* and Babu and Nair (1983) in *Amblypharyngodon chakaiensis*. Spermatogenesis in *Labeo gonius* (Joshi, 1980) and *Covescus plumbeus* (Ahsan, 1966a & b) has been studied. Among the very few Indian marine teleosts which were subjected to similar investigations include, *Hilsa ilisha* (Nair, 1958; Swarup, 1959) and *Mugil cephalus* and *Liza parsia* (Joseph, 1987).

Histological studies of gonad maturation in sillaginids are scanty. In *Sillago ciliata*, Morton (1982) studied gonadal histology and Goodall (1987) have made quantitative histological studies of free and captive populations. Lee Chen - Sheng and Hirano (1985), while discussing the effect of water temperature and photoperiod on the spawning, have worked on the histology of gonads in *Sillago sihama*. Among Indian sillaginids, so far no attempt has been made to investigate this subject. In view of the paucity of works on the histological and cytological studies on gonad development of Indian marine teleosts in general and Sillaginids in particular, the present investigation on the structural organisation of gonads and gametogenesis of *Sillago sihama*, was carried out.

I
OBSERVATIONS

Ovary

The ovary of *Sillago sihama* is of cystovarian type and is surrounded by an ovarian wall enclosing an ovocoel. The ovarian wall consists of an outer layer, *tunica albuginea* and an inner germinal epithelium. The *tunica albuginea* is covered over by a thin layer of peritoneum

(Plate XV Fig. 1) composed of squamous epithelium. *Tunica albuginea* is thickest in immature ovary and thinnest in the ripe one. This layer consists of connective tissue cells, muscle fibres and blood capillaries. Islets of black pigment cells are enclosed in the connective tissue. The germinal epithelium principally consists of a single layer of cuboidal cells which possess very little amount of cytoplasm and relatively large deeply staining nuclei. The germinal epithelium is thrown into finger-shaped folds inside the ovocoel, which are known as ovigerous lamellae. Oocytes of various stages are contained inside the lamellae. The germ cells which are at more advanced advanced stages of maturity are found to be located in the central part of the lamellae and the younger germ cells in the cortical region (Plate XV Fig. 2). With the advancement of maturation, germinal epithelium of the ovary becomes thinner.

In an immature ovary, in addition to the yolk-less immature oocytes, few larger vacuolated oocytes are also seen (Plate XV Fig.3). In a mature ovary, the ovocoel harbours oocytes in different stages of maturity (Plave XV Fig. 4) including the immature oocytes as well as yolk-filled mature oocytes. In the ripe ovary, the germinal epithelium is very thin and ripe oocytes are so closely packed that they practically obliterate the ovocoel and the ovigerous lamellae become indistinct. Along with these ripe oocytes, immature oocytes also occur, which would replenish the yolk-laden oocytes after spawning phase.

Ovarian follicle

During the initial stage of development in the ovary, the oogonia undergo proliferation by mitotic divisions. Then the oogonium enters the prophase of meiosis and will become an oocyte. This process is know as oogenesis. The chromosomes of the oocytes become arrested at the diplotene

PLATE XV.

Fig.1. Transverse section of the immature ovary showing different layers of the ovarian wall and early developing oocytes; Haematoxylin-eosin.

Fig.2. Transverse section of the immature ovary showing ovigerous lamellae containing germ cells in the early stages of development; Haematoxylin-eosin.

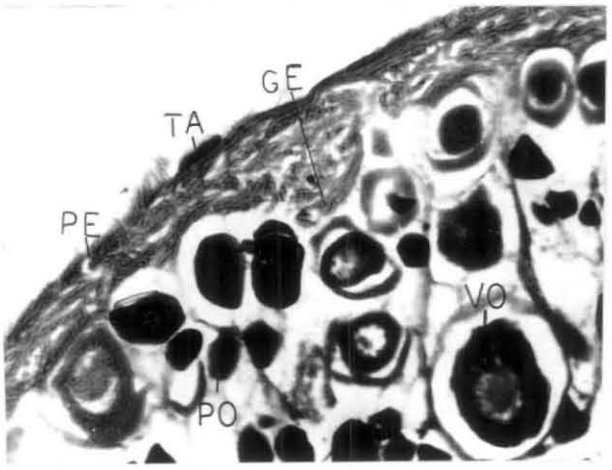
Fig.3. A section of an immature ovary containing Primary oocytes and Vacuolated oocytes; Haematoxylin-eosin.

Fi.g.4. A section of a mature ovary with Primary, Vacuolated and Yolk granule oocytes inside it; Haematoxylin-eosin.

Fig.5. Part of a Tertiary yolk granule oocyte showing various membranes of the follicular epithelium.

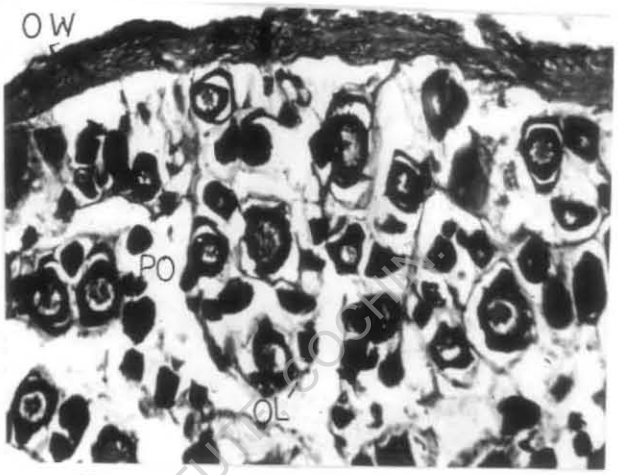
PE-Peritoneum; TA-Tunica albuginea; GE-Germinal epithelium; PO-Primary oocyte; VO-Vacuolated oocyte; OW-Ovarian wall; OL-Ovigerous lamellae; PYGO-Primary yolk granule oocyte; SYGO-Secondary yolk granule oocyte; TYGO-Tertiary yolk granule oocyte; TC-Thecal layer; GR-Granulosa layer; ZRE-Zona radiata externa; ZRI-Zona radiata interna.

PLATE XV



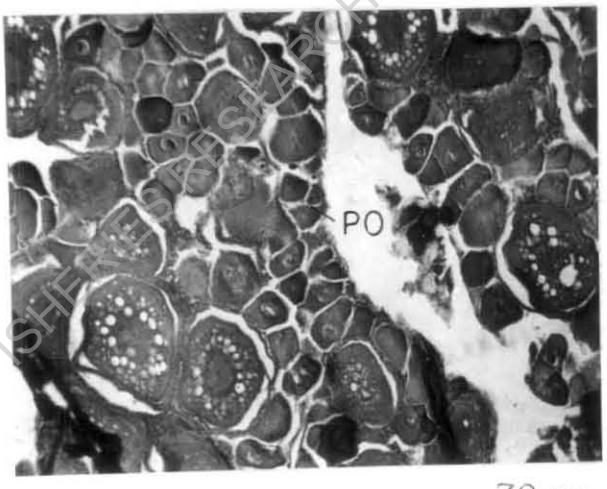
1

70 µm



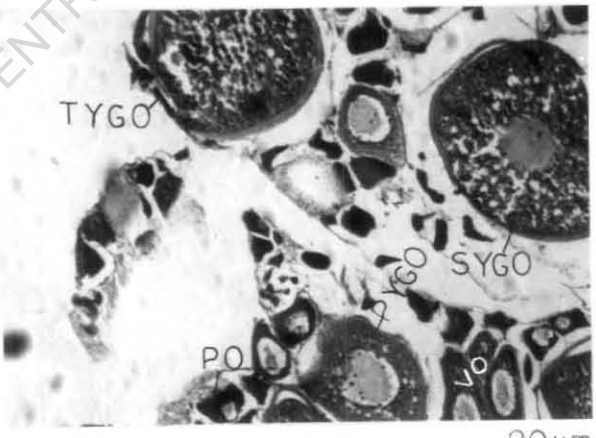
2

70 µm



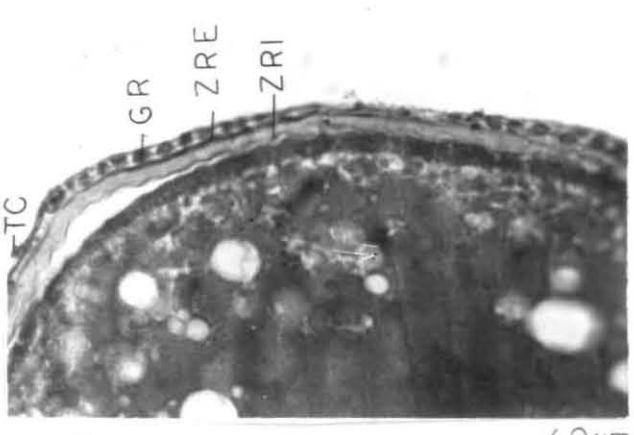
3

70 µm



4

80 µm



5

40 µm

stage and the oocytes enter a period of growth. The oocyte at this stage acquires an investment of epithelial cells. Such units are called follicles. Enlargement of oocytes is caused mainly by the accumulation of yolk.

With the growth of the oocytes, follicle cells multiply and form a continuous follicular layer known as the granulosa layer (Plate XV Fig.5). The granulosa layer consists of nucleated cuboidal follicle cells. Simultaneously, the surrounding stromal connective tissue elements also become organized to form an outer layer of follicular envelope, known as the thecal layer. These two layers are separated from each other by a basement membrane. An acellular layer is formed between the surface of the oocyte and the follicular epithelium. This is variously called as zona pellucida, zona radiata, chorion, vitelline membrane or cortex radiatus, by different authors (Laale, 1980). In the present study, the term zona radiata has been used. Zone radiata becomes prominent in the late secondary yolk granule stage, and exhibits a bipartite structure. The inner finely striated layer is known as zona radiata interna (ZRI) and the outer more homogenous and highly basophilic layer is known as zona radiata externa (ZRE).

Oocyte growth

The growth of oocytes can be divided into two broad phases, namely pre-vitellogenesis and vitellogenesis. During pre-vitellogenesis, the oocyte inclusions show distinct morphological changes preparatory to the formation of yolk. Vitellogenesis is characterised by the synthesis and accumulation of yolk materials.

Several criteria have been adopted for staging the process of oocyte growth, such as size, amount and distribution of various cell inclusions, especially yolk granules, and morphology of chromosome. In the present study, oocyte development has been classified into 7 stages, namely Primary oocyte stage, Vacuolated oocyte stage, Primary yolk granule oocyte stage, Secondary yolk granule oocyte stage, Tertiary yolk granule oocyte stage, Hyaline oocyte stage and Atresia. Atresia has further been classified into 4 stages.

Primary oocyte stage

Primary oocytes are small, sperical or rounded cells found in large numbers. The oocyte, at the beginning of its primary growth phase is referred to as the chromatin-nucleolus stage, with scant cytoplasm and a central nucleus containing a single, large, basophilic nucleolus. The chromatin-nucleolus oocyte has a mean diameter of 37.8 μm. The chromatin threads are moderately basophilic. Concomitant with the growth of the oocyte, the nucleus increases in size and multiple nucleoli are seen along the periphery of the nucleus. The oocyte at this stage is known as perinucleolus oocyte (Plate XVI Fig. 1). The perinucleolus oocyte contains about 9-10 nucleoli. Mean diameter of the perinucleolus oocyte is 69.3 μm and its nucleus has a mean diameter of 23.2 μm. The nucleoli stain deep blue in haematoxylin and deep orange in Mallory's triple stain. During the primary growth phase, the oocyte volume increases markedly and the nucleo-cytoplasmic ratio decreases. The oocyte is enclosed by a thin layer of connective tissue membrane. The follicle layer is not yet apparent.

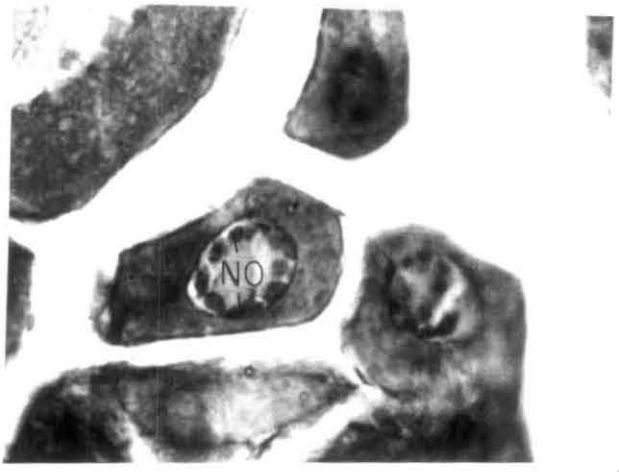
PLATE XVI.

Fig.1. Perinucleolus oocyte; Haematoxylin-eosin

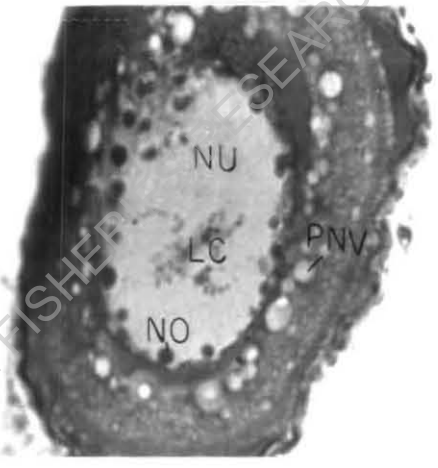
Fig.2. Vacuolated oocyte; Haematoxylin-eosin.

Fig.3. Primary yolk granule oocyte; Mallory's Triple stain

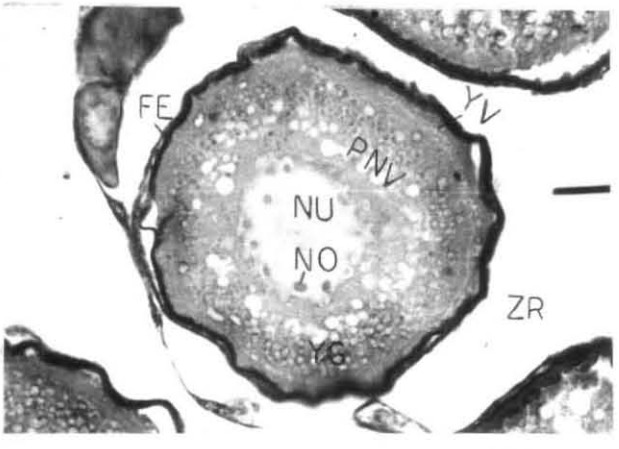
NU-Nucleus; NO-Nucleolus; PNV-Perinuclear vacuoles;
LC-Lampbrush chromosomes; FE-Follicular epithelium;
ZR-Zona radiata; YV-Yolk vesicles; YG-Yolk granules.



1 30 µm



2 20µm



3 80µm

Vacuolated oocyte stage

The earliest sign of yolk accumulation becomes evident in the oocytes measuring about 100 µm in diameter. The nucleus has increased in size, but the nucleo-cytoplasmic ratio has declined. So also there is considerable decline in the basophilia of cytoplasm. In the early vacuolated oocyte, the nucleoli have retained their perinuclear arrangement. Lamp-brush chromosomes could clearly be located in the nucleus of vacuolated oocytes (Plate XVI Fig.2). Nucleus is faintly basophilic. Mean diameter of the nucleus 66.7 µm and that of the nucleolus 2.18 µm. The zona radiata is thin and has a thickness of 1.45 µm. Follicular layer becomes apparent during this stage.

The most characteristic feature of the vacuolated oocyte stage is the appearance of vacuoles of varying size and number in the perinuclear coplasm. They remain unstained in haematoxylin eosin and Mallory's triple stain preparations, when fixed in either Bouin's or NBF fixatives. As the oocyte develops, these vacuoles increase in number and size, gradually filling the cytoplasm from the centre of the oocyte toward the periphery. The size of these vacuoles ranges between 4.35 and 7.25 µ m. The vacuoles situated in the perinculear area are larger than those formed in the peripheral cytoplasm.

The pre-vitellogenic phase in the development of oocyte is represented by the primary oocyte stage and the vacuolated oocyte stage.

The formation of yolk granules marks the beginning of the vitelloginic phase in the growth of oocyte.Depending on the stage of yolk deposition and associated structural changes in the oocyte, the vitellogenic oocyte can be divided into three stages, namely primary, secondary and tertiary yolk granule stages.

Primary yolk granule oocyte stage

During the late vaculoated oocyte stage, the vacuoles have increased in number and size and have arranged themselves as a rather broad ring between the germinal vesicle (nucleus is referred to as germinal vesicle from this stage onwards) and zona radiata. At the same time, another group of vacuoles make their appearance in the cortical ooplasm. These are the yolk vesicles (or cortical alveoli). Later small yolk granules begin to appear in the peripheral cytoplasm close to the zona radiata. This stage is the primary yolk granule oocyte stage (Plate XVI Fig. 3). The yolk granules stain brilliant orange in Mallory's triple stain and red in haematoxylin-eosin preparation. The yolk granules are surrounded by a limiting membrane. Primary yolk granules oocytes have a diameter ranging between 164 and 202 µm. The germinal vesicle has a mean diameter of 79.75 µm. Nuclear membrane appears undulated, with several nucleoli lying in the folds of the membrane. The cytoplasm has lost its basophilia completely. The zone radiata has a mean thickness of 5.01 µm. The follicular layer becomes thick (1.45-2.9 µm in thickness). The granulosa (or follicular epithelium) and theca are apparent at this stage.

Secondary yolk granule stage

Yolk granules increase in number and the yolk layer continues and the layer reaches the surface of the germinal vesicle. The secondary yolk granule oocyte, as it is called now, has a diameter ranging from 214.2 to 252 µm. Nucleus (germinal vesicle) has a mean diameter of 76 .85 µm. Mean diameter of the nucleolus is 2.18 µm. About 11-12 nucleoli are noticed in the nucleoplasm, arranged in a random manner (Plate XVII Fig. 1). The germinal vesicle is

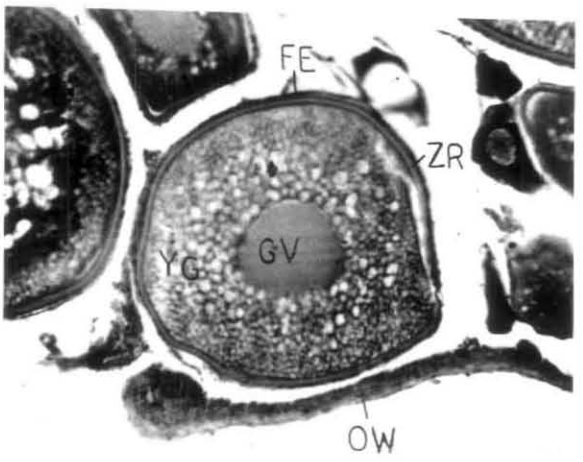
PLATE XVII.

Fig.1. Secondary yolk granule oocyte; Haematoxylin-eosin.

Fig.2. Tertiary yolk granule oocyte; Mallory's Triple stain.

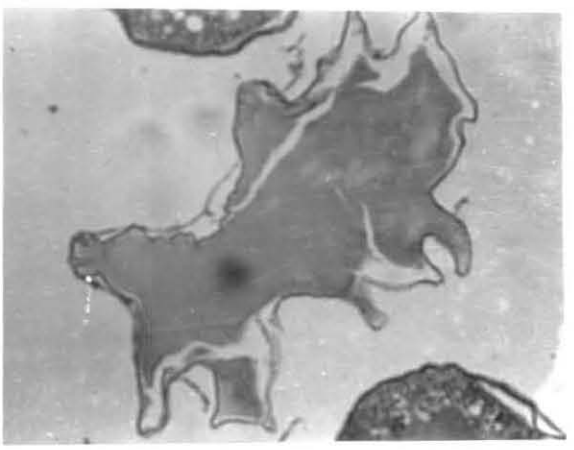
Fig.3. Hyaline oocyte; Haematoxylin-eosin.

OW-Ovarian wall; FE-Follicular epithelium; ZR-Zona radiata; YG-Yolk granules; GV-Germinal vesicle; Y-Yolk mass; OD-Oil droplets.



1 70µm

2 50µm



3 70µm

slightly basophilic and more oval in shape compared to the previous stage. The yolk granules have diameter varying between 2.9 and 8.7 µm. The granulosa layer in the secondary yolk granule oocyte is thicker than that of the primary yolk granle stage. The zona radiata has a mean thickness of 7.25 µm. The zona radiata interna has an undulated appearance and between it and the layer of yolk granules lies a deeply basophilic granular area. Nuclear membrane is inconspicuous.

Teritary yolk granule stage

During the last part of vitellogenesis, referred to as the tertiary yolk granule stage, the yolk granules fuse with one another and form a single mass of yolk. The ooplasm tends to break down in histological sections (Plate XVII Fig. 2). Diameter of the tertiary yolk granule stage range between 239.4 and 264.6 µm. The germinal vesicle is rather difficult to locate. The germinal vesicle has an irregular shape with nucleoli scattered within it. The nuclear membrane disappears, followed by the nucleoli. The zona radiata has further become thickened and measures 8 µm in thickness. As in the case of secondary yolk granule oocytes, the inner layer of zona radiata show inward undulations in the tertiary yolk granule oocytes also. The vacuoles tend to coalesce among themselves. The granular basophilic area between the zone radiata and the peripheral cytoplasm is still present. The follicular epithelium has further thickened and measures about 8 µm in thickness.

Hyaline oocyte stage

Appearance of hyaline oocytes marks the completion of maturation, prior to ovulation and at this stage, there is a rapid increase in the diameter

of the oocyte; the diameter ranges from 283.39 to 350.1 µm. The yolk appears as a homogenous mass filling the interior of the oocyte, so that the latter now appears translucent.

The zona radiata becomes thinner due to the increase in size of the egg. Hyaline oocytes always collapse in histological processing, thus making them look irregular and thereby easily identifiable (Plate XVI Fig. 3).

Atresia

Atresia sets in mainly in yolk granule stages in *S. sihama*. During the present investigation, only few cases of atretic oocytes were observed. Atretic oocyte is characterised by irregular yolk granules and disintegration of zona radiata. The zona radiata appears irregular and eventually ruptures, invasion of the interior of the oocyte begins and the yolk is phagocytosed by the granulosa cells which undergo hypertrophy.

Based on the terminology and description adopted by Davis (1977), atretic oocytes could be classified into four stages in the present study (Plate XVIII Figs. 1-3). They are the following:

The a-stage of atresia: The zona radiata begins to erode and the yolk above its periphery liquifies.

The b-stage of atresia: The zona radiata has completely disintegrated and the yolk continues to liquefy.

The c-stage of atresia: The yolk continues to liquefy. Granulosa is quite prominent and resorbes yolk.

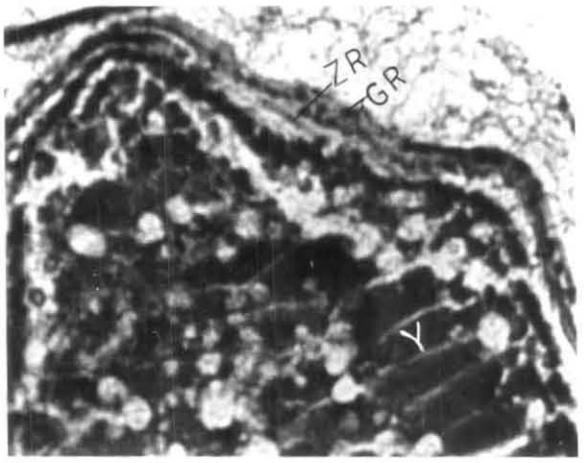
PLATE XVIII.

Fig.1. Transverse section of a stage-a atretic yolk granule oocyte showing disintegration of the zona radiata and hypertrophied granulosa; Haematoxylin-eosin.

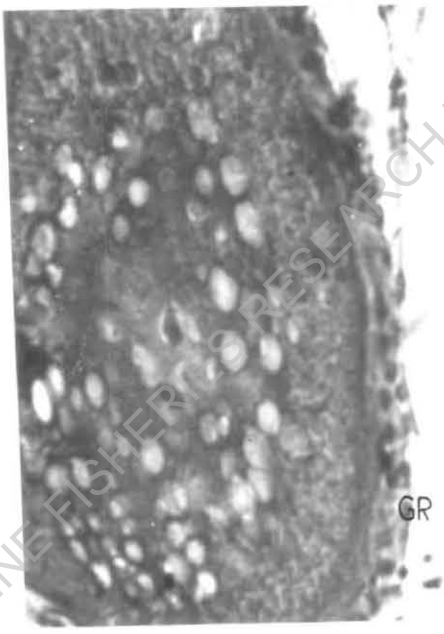
Fig.2. Trasverse section of a stage-b atretic yolk granule oocyte showing considerable hypertrophy of granulosa and liquefying yolk; Haematoxylin-eosin.

Fig.3. Section of a mature ovary showing stage-c and stage-d atretic follicles; Haematoxylin-eosin.

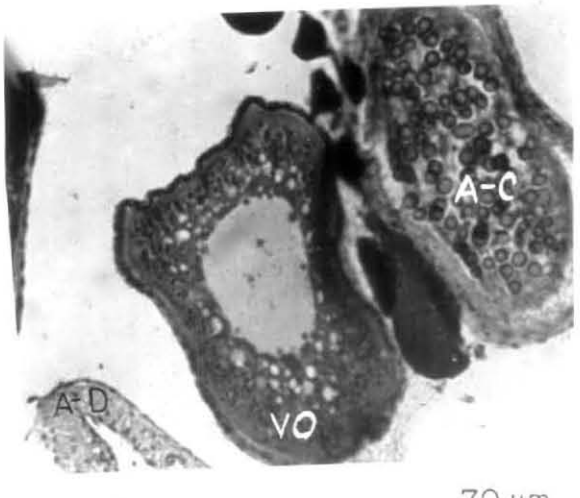
GR-Granulosa layer; ZR-Zona radiata; VO-Vacuolated oocyte; A-C: stage-c atresia; A-D: stage-d atresia.



1 50 µm



2 30µm



3 70µm

The d-stage of atresia: The yolk continues to be resorbed and the granulosa collapses until finally an irregularly shaped body consisting of granulosa and theca remains.

Histological changes during gonadal cycle

The gonadal cycle, which was studied by the conventional methods, such as monthly GSI variations described under chapter IV, was further defined by measurements of oocytes in the histological sections following the method of Harris (1986).

A total of 60 ovaries in various stages of maturation were sectioned and examined during April 1985 to March 1986. The 'horizontal' diameters of 10 randomly selected cells from each of the developmental stages of oocytes were measured using ocular micrometer. This technique produced consistent results in the vacuolated oocyte and yolk granule oocyte stages, but variation was substantial in the primary, hyaline and atretic oocytes owing to their irregular shapes. The diameters of 10 of the largest oocytes in each of these ovarian sections were then measured to find the mean diameter of large oocytes. The frequency of various oocyte development stages was determined by measuring all oocytes present in one or more microscopic fields of view. Then they were classified and counted until a total exceeding 100 cells had been reached. Thus, oocytes from 10 ovaries of stage I, 15 ovaries of stage II, 17 ovaries of stage III, 15 ovaries of stage IV and 3 ovaries of stage V were measured. Mean and standard deviations of the percentage frequency of various stages of oocytes in the ovaries are given in Table 27.

It may be seen from the Table 27 that the primary oocytes are the dominant stage in all maturity stages of the ovary. They show gradual decline

from 95 percent in stage I to 51.8 percent in stage IV and further increase to 62.7 percent in stage V. The vacuolated oocytes, after a sharp increase in stage II, decrease to 13.6 percent in the ripe ovary and further increase in stage V. The yolk granule oocytes make their appearance in stage II and maximum percent of 27.5 is shown in stage III. Since then they decline in proportion in the two subsequent stages. Hyaline oocytes appear in stage IV ovary and in partially spent ovary (stage V) they represent 2.8 percent. Atretic oocytes increase gradually from stage III to stage V.

TABLE 27. Mean percentage frequency of oocyte stages

Maturity stage of the ovary	Number of ovaries examined	Mean ± SD of percentage frequency						
		Primary oocytes	Vacuolated oocytes	Yolk granule oocytes			Hyaline oocytes	Atretic oocytes
				Primary	Secondry	Tertiary		
I	10	95.0± 14.5	5.0± 1.7	-	-	-	-	-
II	15	57.4± 17.5	32.0± 6.4	5.5± 1.2	5.1 1.2	-	-	-
III	17	54.7 ± 16.4	16.5 ± 5.3	12.7 ± 6.3	7.7 ± 1.3	7.1 ± 1.2	-	1.3 ± 1.0
IV	15	51.8 ± 14.5	13.6 ± 2.4	7.5 ± 3.3	4.5 ± 1.3	2.7 ± 1.2	18.1 ± 7.5	1.8 ± 1.5
V	3	62.7 ± 15.6	24.8 ± 6.7	4.5 ± 0.5	3.1 ± 0.8	-	2.8 ± 2.5	2.1 ± 1.5

The mean diameter of large oocytes in histological sections rose gradually from June, 1985 to January, 1986 (Plate XIX, Fig. 1). Large standard deviations during the spawning season, i.e., July, 1985 to February, 1986 are due to the presence of ovaries in stages IV and V, which have all the oocyte groups in them.

The relative volume (expressed as the square root of the product of mean volume of each oocyte stage and its percentage frequency) occupied by each oocyte stage in each of five gonad maturity stages is given in Plate XIX, Fig. 2. This indicated the relative importance of oocyte types at different stages of maturity. Primary oocytes though remained at a high frequency, showed a low relative volume. Relative volume of yolk granule oocytes and hylaine oocytes were maximum in stages III and IV, respectively. Relative volume of atretic oocytes was comparatively higher in stage IV and V.

Testis

In the transverse section (Plate XX A), the mature testis appears to be kidney-shaped with the vas deferens situated in its concavity. The vas deferens which runs throughout the entire length of each testis along its inner lateral side, gives rise to a number of primary and secondary ducts (vasa efferentia) that branch into the body of the testes. The terminal end of each vas efferens ends in a seminiferous lobule. Each lobule has central cavity that is continuous with the lumen of the vas efferens.

All along the inner wall of the louble are germinal cysts containing germ cell, namely the primordial germ cells, the spermatogonia, the spermatocytes, the spermatids and the spermatozoa in various stages of development.

PLATE XIX

Fig.1. Monthly mean diameter of 10 largest ova in the ovarian sections of *S. sihama*.

Fig.2. Relative volume of each oocyte in the five ovarian maturity stages of *S. sihama*.

PLATE XIX

Fig.1

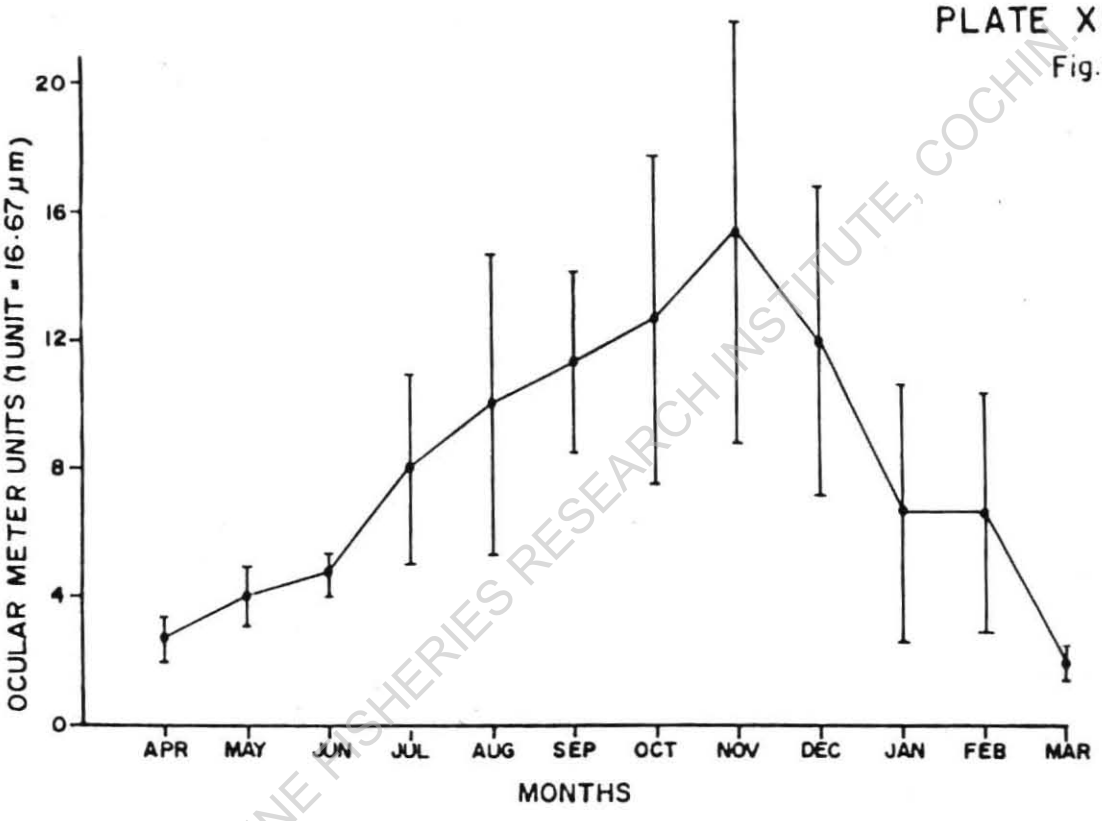


Fig.2

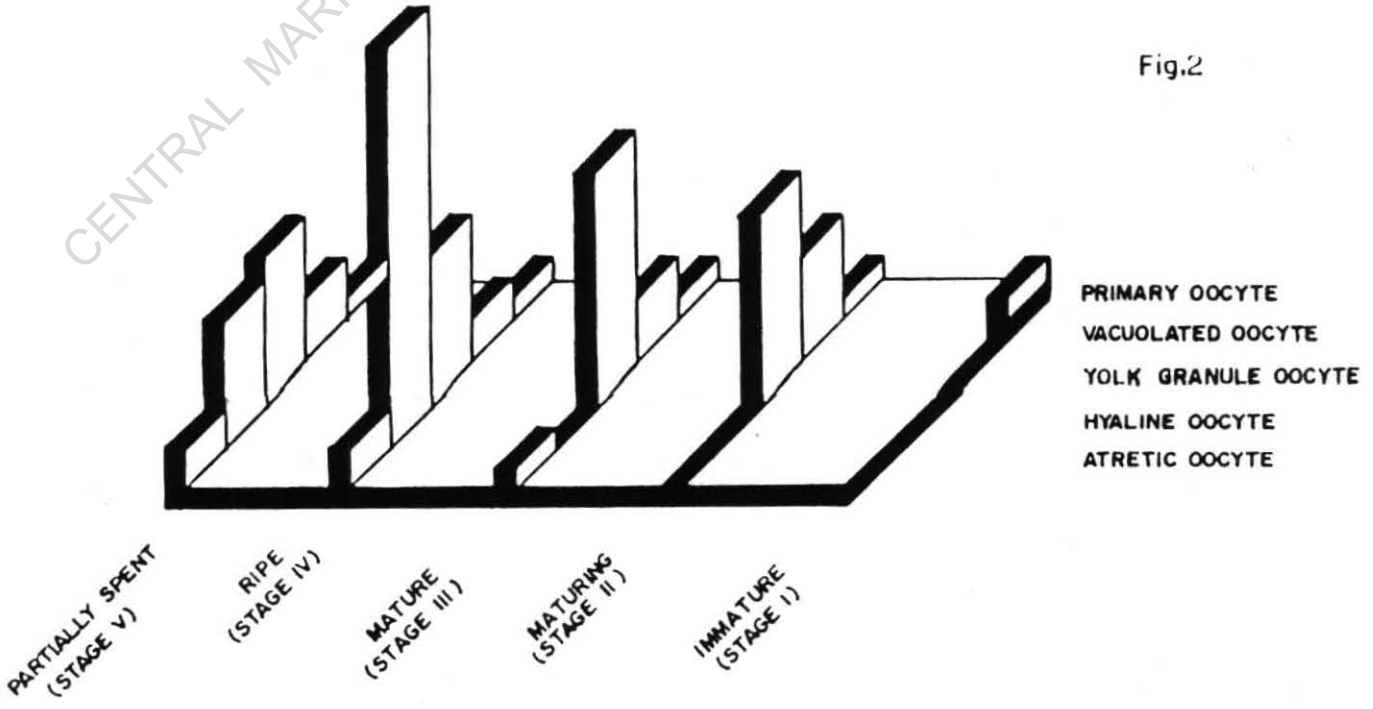


PLATE XX A

Diagramatic representation of the cross section of mature testes of *S. sihama*.

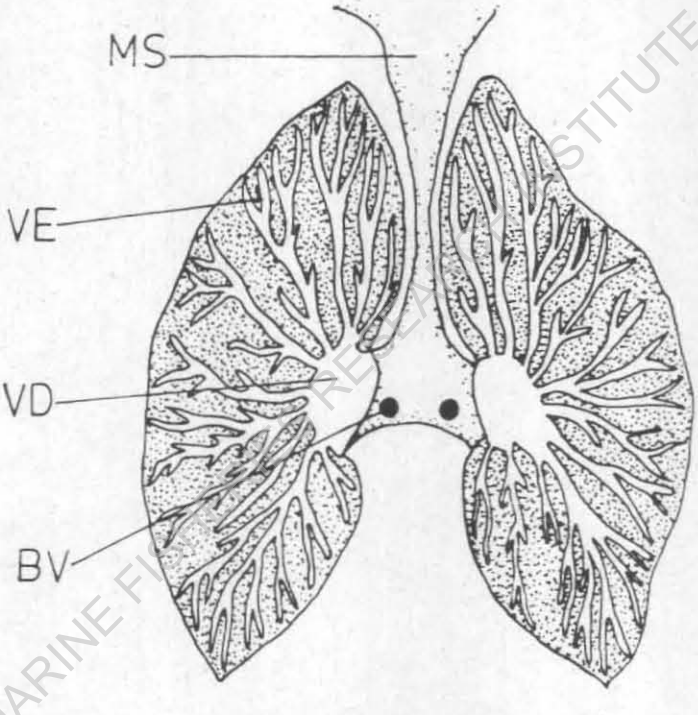
MS-Mesentery; VE-Vas efferens; VD-Vas deferens; BV-Blood vessel.

PLATE XX B

Transverse section of a mature testis showing various parts of a seminiferous lobule; Mallory's Triple stain.

CW-Connective tissue wall; IT-Interlobular tissue; LC-Leydig cell; PS-Primary spermatocyte; SS-Secondary spermatocyte; SD-Spermatid; SH-Sperm head; ST-Sperm tail; SZ-Spermatozoa.

PLATE XX A



The seminiferous lobules are separated from one another by the basement membrane and inter-lobular somatic tissue. Immediately outside the basement membrane lies discontinuous row of spindle-shaped cells (Boundary cells). A few connective tissue cells, blood vessels and Leydig cells are also seen in the inter-lobular space. The leydig cells are large polygonal cells seen in the interlobular somatic tissue at the junction of two or three seminiferous lobules (Plate XX B).

The entire body of the testes is protected externally by a connective tissue capsule known as the *tunica albuginea*.

The germinal cysts containing germ cells undergo divisions. The spermatogonia, formed after mitotic divisions of the primordial germ cells, in turn undergo numerous mitotic divisions and result in the formation of primary spermatocytes. The first meiotic division of the primary spermatocyte produces two daughter cells, the secondary spermatocytes. The secondary spermatocytes then transform into spermatids through the second meiotic division. These spermatids, despite the presence of haploid set of chromosomes, cannot function as male gametes, but have to undergo differentiation, a process termed as Spermiogenesis. As a result, the spermatozoa are formed. Major changes taking place during the transformation of spermatid to spermatozoa are a reorganization of nucleus and cytoplasm as well as the development of flagellum; no cell division occurs during this process. Thus the number of spermatozoa formed at the end of spermiogenesis is the same as the total number of spermatids present.

The different stages of spermatogenesis and spermiogenesis are distinguished on the basis of size of the cells, the nuclear charactertistics, and the cytoplasmic

morphology. The characteristic features of each of these stages of *S. sihama* are given below:

Primordial germ cells (Stem spermatogonia)

The primordial germ cells occur in interstitial areas forming new lobules and in the walls of the existing lobules. These cells are irregular in out line. The nucleus has a mean diameter of 4.7 μ m and stains lightly.

Spermatogonia

The spermatogonia are intially smaller than the primordial germ cells and the nucleus has a mean diameter of 4.5 μm. But they undergo a period of growth and become the largest of all the spermatogenic cells, with the nucleus measuring 5.1 μm in diameter. Staining intensity of the nucleus increases in the larger spermatogonia. The nucleus is ovoid or rounded and contains one or two nucleoli. Spermatogonia are often observed in nests attached to the lobule wall.

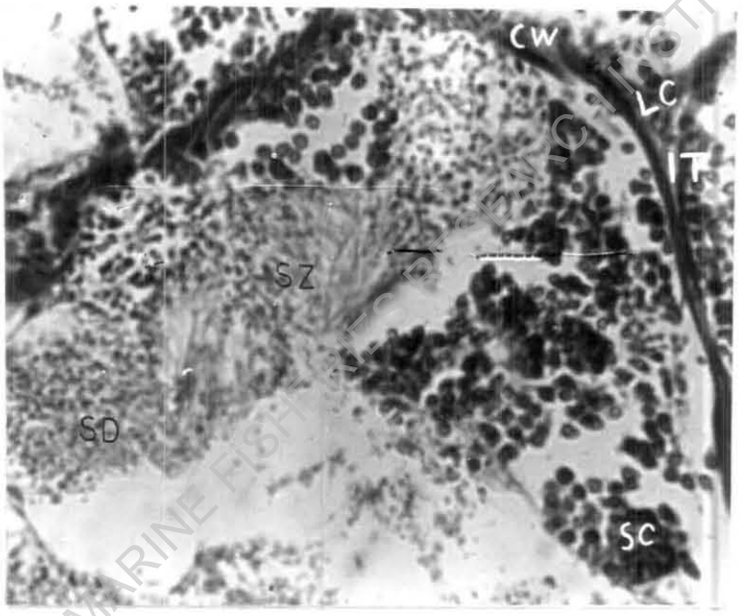
Primary spermtocyte

Mean diameter of the primary spermatocyte nucleus is 3.3 μm. They are formed by mitotic division of spermatogonia and occur in nests on the inner side of the lobule wall. These cells have little cytoplasm. Nucleus contains dense chromatin material as evidenced by deep staining property. Nucleus stains intense blue in Mallory's triple preparation.

Secondary spermatocyte

The secondary spermatocytes are formed by the meiotic division of the primary spermatocytes. They appear fewer in number since they develop to the next stage rapidly. The chromatin of the nucleus is dense and mean nucleus diameter is 1.8 μm. There is little or no cytoplasm and the cells are found in

PLATE XX B



30 µm

PLATE XX B

Transverse section of a mature testis showing various parts of a seminiferous lobule; Mallory's Triple stain.

CW-Connective tissue wall; IT-Interlobular tissue; LC-Leydig cell; PS-Primary spermatocyte; SS-Secondary spermatocyte; SD-Spermatid; SH-Sperm head; ST-Sperm tail; SZ-Spermatozoa.

nests extending into the lobule lumen. It is noticed that the secondary spermatocyte nucleus is more circular in out line than that of the primary spermatocyte nucleus. Nucleus of the secondary spermatocyte also stains deep blue in Mallory's triple preparation.

Spermatids

Spermatids are formed by the last cell division in the sequence spermatogenesis. They have no distinguishable cytoplasm and mean nucleus diameter is 1.57 µm. In Mallory's triple preparation, the spermatids stain deep orange indicating the presence of dense chromatin material. Like other spermatogenic stages described above, spermatids too remain in clusters after detachment from the lobule wall.

Spermatozoa

Spermatozoa are fully developed gametes lying freely in the lumen of the seminiferous lobule. Their pear-shaped heads have a mean width of 1.2 µm and stain deep orange in Mallory's triple preparation. The spermatozoa often retain their organization into 'parachute-shaped' clumps due to adhesion of the sperm tails.

The histological features of the testes of *S. sihama* during different stages of maturity are as follows:

Stage I (Immature) : In the longitudinal section, the immature testis is found to be made of connective tissue stroma in which isolated nests of large irregular primordial germ cells are distributed. Some spermatogonial cysts are also found. In the late immature stage, seminiferous lobules become visible, containing spermatogonia and dividing spermatocytes (Plate XXI, Fig. 1 & 2).

PLATE XXI

Fig.1. Longitudinal section of immature (early) testis; Haematoxylin-eosin.

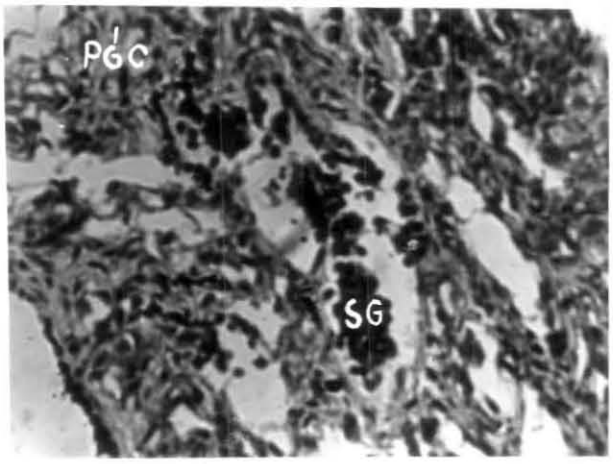
Fig.2. Longitudinal section of immature (late) testis; Haematoxylin-eosin.

Fig.3. Transverse section of maturing (early) testis; Mercuric bromophenol blue.

Fig.4. Transverse section of maturing (late) testis; Haematoxylin-eosin.

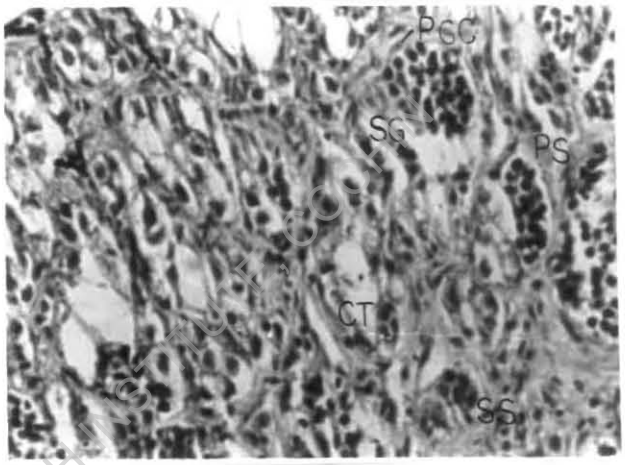
SG-Spermatogonia; PGC-Primordial germ cell; CT-Connective tissue; PS-Primary spermatocyte; SS-Secondary spermatocyte; SD-Spermatid; IT-Interlobular tissue; LU-Lumen; SZ-Spermatozoa.

PLATE XXI



1

30 µm



2

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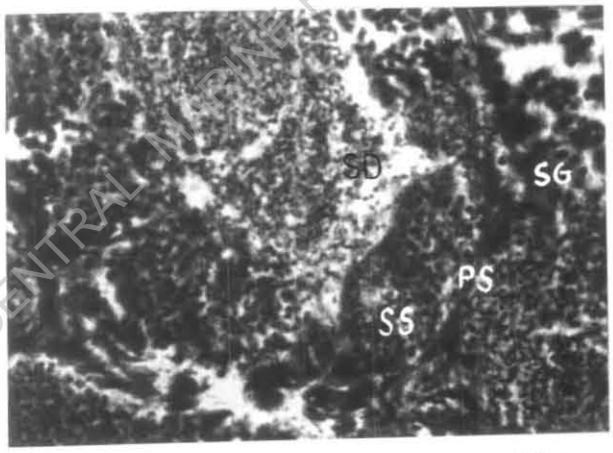

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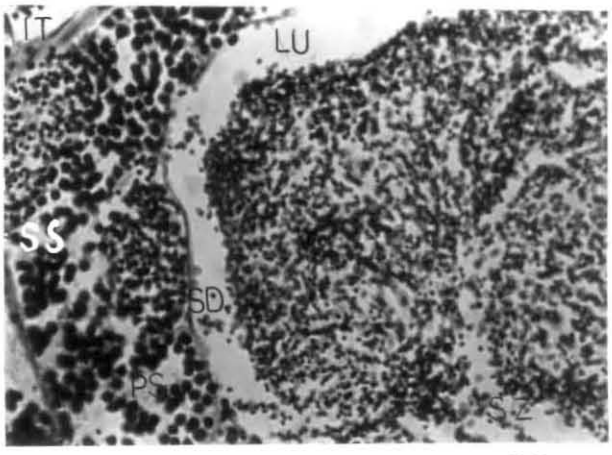


4

30 µm

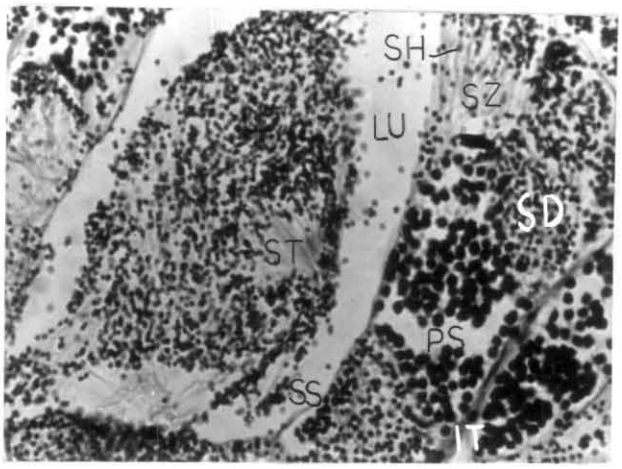
PLATE XXII

Fig.1. Transverse section of a mature testis - 'parachute' like spermatozoa are visible; Haematoxylin-eosin.

Fig.2. A seminiferous lobule of Oozing testis filled with spermatozoa; Mallory's Triple stain.

Fig.3. Transverse section of a Partially spent testis - lobules partially empty of spermatozoa are visible; Mallory's Triple stain.

IT-Interlobular tissue; LU-Lumen; PS-Primary spermatocyte; SS-Secondary spermatoxyte; SD-Spermatid; SH-Sperm head; ST-Sperm tail; SZ-Spermatozoa; SC-Spermatoxyte.



1

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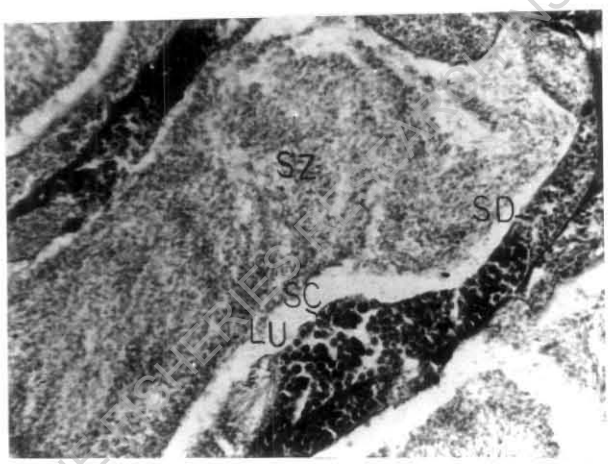
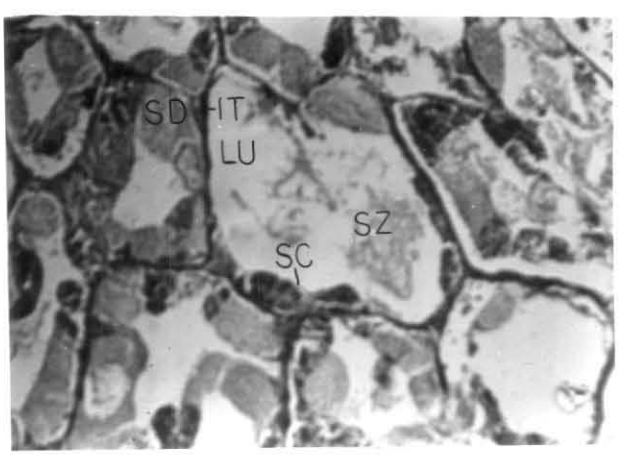


2

30 µm



3

100 µm

Stage II (Maturing): Spermatogenic activity is high during this stage (Plave XXI, Fig. 3). The lobules increase in their size. Very few spernatogonia are located in the lobules, while compactly arranged seminiferous cysts of actively dividing spermatocytes and spermatids are found. Spermatogonia can also be seen. In the advanced maturing stage, an interlobular lumen becomes visible in most of the seminiferous lobules (Plate XXI Fig.4).

Stage III (Mature): As in the previous stage, the spermtogenic activity continues to be very high in this stage. The spermatozoa exhibit the 'parachute like' arrangement in some regions, while in others they are liberated into the lumen of the seminiferous lobule (Plate XXII, Fig. 1).

Stage IV (Oozing): Seminiferous cysts are reduced in size and number and are found along the periphery of the lobules. At this stage, the lumen of the lobule is very much enlarged and filled with free, motile spermatozoa (Plate XXII, Fig. 2). These spermatozoa are no longer arranged in clusters. The interlobular somatic tissue is highly reduced.

Stage V (Partially spent): The lumen of several seminiferous lobules contain large empty spaces lined by seminiferous cysts of developing spermatocytes. A few degenerating residual spermatozoa are also found in the lumen on these lobules (Plate XXII, Fig.3).

DISCUSSION

Gonadal development in bony fishes differs from that of other vertebrates. In higher vertebrates, the gonads consist of a peripheral cortex formed by a thickened germinal epithelium and a central medulla, separated by a tunica albuginea. The primordial germ cells appear at first in the cortical region. In the bony fishes, there is only a single primordium which corresponds to this cortical region, and is derived more from the peritoneal epithelium than from the measonephric blastula (d'Ancona, 1950, 1952, Dodd, 1965).

Ovary

Early in development, longitudinal ridges arise on the ventrolateral surface of the developing ovaries, and fuse to enclose a cavity which is purely coelomic and is lined by the peritoneal epithelium, unlike the ovarian cavities of other vertebrates, which are lined by mesenchyme. In teleostei and Lepidostei, the cavity persists, giving rise to the cystovarian condition, where as in Dipnoi, Chondrostei and *Amia*, among Holostei, the ovary is solid and uncovered. The ovary of *S. sihama* is typically cystovarian type. The cystovarian ovaries of this fish have posterior extensions of their wall and cavities which form oviduct and these open into the cloaca. In some other teleosts, the oviducts degenerate and ova are released into the body cavity, not into the ovarian lumen. The ovarian cavity has been regarded as a mere "stock room" in which ovulated eggs are kept temporarily until they are spawned. But in some species, like medaka, the lining of the cavity has been assigned with secretory activity.

The so called germinal epithelium appears to be peritoneal in origin, there being no evidence that the germ cells originate from anything other than the primordial gonia. However, this aspect is much debated. While Mendoza (1943), Rai (1967) Trom-Blom (1959), Bara (1960) and Aravindan and Padmanabhan (1972) felt that new crop of oogonia originate from the germinal epithelium, other workers, including Honma and Tamura (1962), Belsare (1962), Braekevelt and McMillan (1967), Khanna and Sanwal (1971) and Shrestha and Khanna (1979) believe that the new crop of oogonia arise from the residual oogonia.

In *S. sihama,* the germinal epithelium consists of a single layer of cuboidal cells with little cytoplasm and deeply staining nuclei. Tunica albuginea was thickest in immature ovaries and thinnest in ripe ones. The stroma seemed to be relatively sparse and consisted mainly of connective tissue which supports the ovigerous lamellae and carries appreciable vasculature and nerve supply to the follicles.

Oocyte growth

The primary oocytes formed by the first mitotic division of oogonia undergo meiosis to yield, first, a secondary (haploid) oocyte and a first polar body, and subsequently by mitotic division of the secondary oocyte, the definitive gamet (ovum) and a second polar body. Oogenesis, thus, is dependent in the first place upon the mitotic division of the primordial germ cells and oogonia. The chromosomal changes characteristic of meiosis occur only in the Primary oocytes that have been formed by oogonia.

It should be noted that the term oogenesis has frequently been used incorrectly in the past, and occasionally still is, to apply to the growth of the oocyte and its follicular envelope, associated in some species with vitellogenesis processes which are known to occur after part of the meiotic prophase has taken place (Zuckerman and Baker, 1977). These workers comment that it becomes too vague a term if it is used to comprehend matters such as the seggregation of the germinal elements in the blastula, their migration to the genital ridges and stages in cytological maturation of the primordial oogonia and oocytes. In the present work, the word oogenesis has been avoided, to be replaced by 'oocyte growth', since the study was concentrated on the growth of oocyte and its follicular envelope.

The development of oocytes in all teleosts is basically the same, with slight differences in yolk composition, yolk deposition, rapidity of growth and surrounding membranes (Lal, 1963). The early oocyte has a large nucleus and small amount of basophilic cytoplasm. Growth is due mainly to an increase in non-yolky cytoplasm until primary and secondary yolk are laid down.

In the present work, the sequence of oocyte maturation was divided into seven stages and the process of atresia into four stages. The stages such as Chromatin nucleolus stage, perinucleolus stages, described by Yamamoto (1956a), were grouped under 'Primary oocyte stage'. The 'vacuolated oocyte stage' corresponds to the 'Yolk vesicle stage' in Yamamoto's (1956a) classification. These two terms exist because of the difference in the initiation of yolk formation in different fish species. 'Vacuolate oocyte', as used in the present study, is one in which yolk appears in the form of vacuoles in the

perinuclear cytoplasm. On the other hand, in 'yolk vesicle oocyte', which is described in several species, vacuoles or cortical alveoli make their appearance in the peripheral ooplasm. The term 'hyaline' oocyte, which corresponds to the 'ripe' stage in Yamamoto's classification was used in other fishes such as *Trachurus trachurus* (Macer, 1974), *Limanda limanda* (Htun-Han, 1978a) and *Sillago ciliata* (Goodall *et al.*, 1987). The 'migratory nucleus stage' was not frequently seen in the sections and hence not included in the present classification. The difficulty in locating 'migratory nucleus oocyte', has been expressed by other workers like Davis (1977), who remarks, "detection of this stage depended upon sectioning through the nucleus, a rare occurrence in oocytes with extensive yolk". Further, he commented that this stage was of limited use in determining the maturation stage of the ovary in *Tandanus tandanus*. The four stages of atresia have been described after Davis (1977).

Follicle wall

In their development, structure and composition, the follicular wall shows great diversity among teleosts (Guraya, 1986). In several species few follicle cells develop around the young oocytes (Chaudhry, 1956; Jollie and Jollie, 1964; Anderson, 1967; Flugel, 1964, 1967a; Rastogi, 1970; Guraya *et al.*, 1975; Shackley and King, 1977; Guraya, 1978; Brusle, 1980). In the present work, follicle cells appeared for the first time around the vacuolated oocytes. The primary oocytes were surrounded only by a thin layer of connective tissue membrane. As the oocyte grows, the follicle cells increase in number, probably by mitosis to constitute a continuous follicular epithelium which retain its single layered structure throughout oocyte growth as was observed

in the fish studied in the present work. A follicular epithelium of two cell thickness has been described in *Fundulus heteroclitus* (Anderson, 1966). In some species such as *Tilapia thollori* and *Arius thalassinus*, the follicular epithelium exhibits a pseudo-stratified appearance which disappears during advanced maturity stages (Kraft and Peters, 1963; Gabaeva and Ermolina, 1972; Guraya, 1978). Probably this pseudostratified condition of the follicle wall indicates the presence of cellular reserve which could be utilized for the rapid growth of the oocyte. Such a condition was not seen in the present material.

With oocyte growth, the follicle layer (also known as the 'granulosa layer') thickened considerably in the oocytes of *S. sihama*. The maximum thickness of 8 µm was attained in the Tertiary yolk granule stage. The follicle cells also undergo changes in their morphology with oocyte development. In the vacuolated stage, the follicle cells were less conspicuous with either spindle shaped or squamous appearance. As the oocyte developed to Tertiary yolk granule stage, the follicle cells were quite prominent, with dark staining nuclei and rather cuboidal in outline. As the follicular epithelium gets stretched, the amount of cytoplasm in the follicle cells will be decreased.

The poor development of follicle cells in the oocyte prior to vitellogenesis may suggest that they do not play a role in synthesising essential materials which may be required for building- up process in the oocyte. It is likely that rather the germinal vesicle may contribute toward this need as evidenced by the multiplication of nucleoli. An exactly opposite situation exists in the higher vertebrates, where the oocytes are covered by large follicle cells, with the germinal vesicle not developing numerous nucleoli. Generally

there will be only one nucleolus inside the nucleus apart from other small nucleolar like bodies.

Zona radiata

Zona radiata forms an acellular layer laid between the surface of oocyte and follicular epithelium. It shows great diversity in its structure and chemistry in different groups of fishes.

Depending on the species as well as on the stage of oocyte maturation in the same species, zona radiata shows variable numbers of layers or zones, thus forming either a monopartite, bipartite or tripartite acellular envelope between the oocyte surface and follicular epithelium (Guraya, 1986). In their width, structure and texture, its different zones vary greatly in different teleosts as revealed with electron microscope.

In several teleosts, zona radiata of the oocyte has been reported to be made up of a striated inner layer, zona radiata interna and a relatively homogenous outer layer, zona pellucida proper (or zona radiata externa) (Hurley and Fischer 1966; Flugel, 1967a; Riehl, 1976b, 1978b; Flegler, 1977; Riehl and Schulte, 1977a; Erhardt, 1978; Mayer *et al.*, 1988). The striations on the inner layer are known to represent the microvilli of the oocyte surface traversing the zona radiata (Kraft and Peters, 1963; Guraya, 1965; Guraya *et al.*,1975, 1977). In the present work also, the zona radiata of the late secondary yolk granule and tertiary yolk granule oocytes was found to show a bipartite structure, with a faintly striated inner layer (ZRI) and a homogenous and highly basophilic outer layer (ZRE).

The basophilic granular area observed between the oocyte surface and the zona radiata in the oocytes of advanced maturation in the present material, was also reported in *Mugil cephalus* (Pieu po-Chung and Chiu Lias,1975). In the advanced oocytes of *Gadus*, Gokhale (1957) found a darker cortical zone and lighter inner zone. A granular 'zonoid' layer was found in the early oocytes of some teleost fishes (His, 1873; Brock, 1878; Scharff, 1888). The actual significance of this granular layer is not known. Wheeler (1924) has asserted that the zonation of ooplasm is an artifact produced by fixation.

Zona radiata undergoes changes during ovum maturation and ovulation. In the present study, the zona radiata has a mean thickness of 1.45 μm in the vacuolated oocytes, 5.01 μm in the primary yolk granule oocytes, 7.25 μm in the secondary yolk granule oocytes and 8 μm in the tertiary yolk granule oocytes. In the hyaline oocytes, the zona radiata was very thin. It appears that the thinning of the zona radiata is partially a result of stretching the envelope when the egg increases in diameter (Guraya, 1986).

Thecal layer

Theca forms a connective tissue layer lying outside the basal lamina, which in turn is lying outside the follicle layer. In *S. sihama*, the thecal layer was poorly defined in the oocytes irrespective of the stage of development. Guraya *et al.* (1965) have also found that in *Channa*, the theca was poorly developed. Normally this layer is composed of collagenous fibres, capillary loops and fibroblast- like (or stromal) cells (Hurley and Fischer,1966).

Yolk elements

The cytoplasm of the late perinucleolus stage oocytes in *S. sihama*, was found to contain minute vacuoles surrounding the nucleus. These minute vacuoles gradually increased in size and number and the oocyte would become a 'vacuolated oocyte'. Appearance of this stage marked the beginning of yolk formation in this species. This was found to be followed by the appearance of small highly eosinophilic granules and another set of vacuolar bodies in the peripheral ooplasm. The vacuoles seen during the early part of the oocyte development in the perinuclear ooplasm are the oil droplets as revealed in histochemical tests. The contents of these droplets are lost during the routine histological sectioning and hence appear in the form of empty vacuoles. The set of vacuolar bodies formed simultaneously with the yolk granules are the yolk vesicles or cortical alveoli.

The sequence of the formation of vitelline elements in teleost fish oocytes is known to vary among different species. In *Plecoglossus altivilis*, Matsuyama and Matsuura (1982) observed the formation of three vitelline elements, namely yolk vesicles, yolk globules and droplets of fat in that order. In Japanese eel, *Anguilla japonica*, Yamamoto *et al.* (1974) found that oil droplets form for the first time in the perinulcear area followed by the formation of yolk vesicles. Soon after the formation of yolk vesicles, yolk globules began to appear in the peripheral part of the cytoplasm. Yolk vesicles are reported to form before oil droplets in the oocyte development of *Oncorhynchus masou* (Yamamoto *et al.*,1959) and *Salmo gairdneri* (Yamamoto *et al.*,1965). The lipid droplets of the smelt, *Hypomesus japonicus* appear after the formation of both yolk vesicles and globules (Yamamoto,1956c)

Oil droplets were not detected at any stage of oocyte development in the oocytes of *Tandanus tandanus* (Davis, 1977) and gold fish *Carassius auratus* (Yamamoto and Yamazaki, 1961; Khoo, 1979).

In a large number of teleost fishes, the yolk vesicles make their appearance in the peripheral ooplasm from where their formation spreads toward the inner ooplasm and finally aggregate in the cortical ooplasm to constitute a conspicuous zone. The yolk vesicles are also described as cortical vacuoles, vacuolar yolk, intravacuolar yolk, intravesicular yolk, carbohydrate yolk, vacuome, etc. (Malone and Hisaoka, 1963; Guraya, 1965, 1982; Ginsburg, 1968; Shahi *et al.*,1979). In the present work, though the yolk vesicles appeared in the peripheral ooplasm, they did not spread centripetally, but shifted more toward the cortical region of the oocyte.

Appearance of a highly basophilic peri-nuclear complex, known as 'yolk nucleus' or Balbiabi's vitelline body, in the previtellogenic oocytes of several teleost fish species has been reported (Chaudhry, 1952; Stolk, 1959; Port and Zahnd, 1962; Kumari and Padmanabhan, 1976; Guraya *et al.*1965; Kapoor, 1977; Sobhana and Nair, 1977; Shahi *et al.*, 1979; Choudhery *et al.*1979; Takahashi, 1981). The yolk nucleus substance is presumed to be the basic cytoplasmic machinery for various synthetic activities in the oocyte. In the present study, such a structure could not be observed at any stage of oocyte development. In *Lepidocephalus thermalis*, Ritakumari and Nair (1979) also did not find yolk nucleus during oocyte development.

The initial formation of yolk granules in the oocytes of *S. sihama* was similar to that observed in other fishes. As the maturation of oocyte advanced, the yolk granules started developing centripetally from the peripheral ooplasm and have undergone fusion. The fusion of yolk granules

would positively accelerate water absorption of oocyte to bring about ovulation. It is assumed that yolk granule fusion is induced essentially by some intracellular changes of oocytes during meiosis and it may be influenced by the chemical composition of the ovarian fluid, supposed to fluctuate concurrently to some extent (Oshiro and Ibiya, 1982). Depending on the state of yolk granules in the late phase of vitellogenesis, the mass of yolk has been called as either 'continuous yolk mass' or 'non-continuous yolk mass' (Yamamoto and Yamazaki, 1961). In 'continuous yolk mass' type, the yolk globules fuse together to form a single yolk mass at the end of vitellogenesis as seen in some teleosts (Marza *et al.*, 1937; Yamamoto *et al.* 1965). In several other fish species, the yolk globule fusion, though do occur, will not result in the formation of a single mass of yolk (Yamamoto, 1958; Belsare, 1962; Barr, 1963a; Davis, 1977; Rai, 1967; Dixit and Agrawala, 1974; Guraya *et al.* 1975; Konopacka, 1935; Narain 1937; Mas, 1952).

In the present study, it has been seen that yolk granules undergo fusion and their mean diameter was 5.7 µm in the concluding stages of vitellogenesis. In the tertiary yolk granule oocyte, coalesced yolk of variable size appeared particularly in the central region. At the same time, the oil droplets have also fused and in the histological section, large vacuoles could be seen in the ooplasm. In hyaline oocyte, yolk appeared as homogenous mass. Considerable extent of fluid absorption occurs during this stage, which decreases the specific gravity of the egg, thus permitting it to float when shed. The fluid absorption would be accompanied by a clearing of the contents of the oocyte and the disappearance of the germinal vesicle, thus rendering the oocyte transparent. As per the classification of Yamamoto and Yamazaki (1961), thus, the yolk mass formation in *S. sihama* may be belong to the 'continuous' type.

Germinal vesicle

The chromatin-nucleolus oocyte in the ovary of *S.sihama* has a large nucleus and little amount of cytoplasm. As oocyte growth progressed, the cytoplasm increased in volume and nucleus became enlarged to form the germinal vesicle. The morphological changes taking place in the germinal vesicle during previtellogenesis and vitellogenesis are more or less similar in different teleosts, suggesting the presence of similar regulatory mechanisms(Guraja, 1986).

The early differentiation of the primary oocyte to the vacuolated oocyte in *S. sihama* was characterised by the nuclear membrane becoming wavy or undulated. These undulations of the nuclear membrane were seen till the secondary yolk granule oocyte stage. Obviously these undulations and folds increase the relative surface of the germinal vesicle for nucleocytoplasmic exchanges of molecules during oocyte growth. However, regulation of these morphological alterations of nuclear envelope is not known. Two processes apparently affect the nuclear membrane during oocyte development. One is concerned with the partial or complete perforation of the envelope, possibly facilitating the passage of nuclear material into the cytoplasm and the other is the enhancement of the nuclear membrane area as well as its area relative to nuclear volume.

The nuclear membranes are reported to form 'blebs', which are pinched off so as to become detatched from the nuclear envelope (Scharrer and Wurzelmann, 1969 a,b). In the present work, a similar observation was made. A careful examination of the nuclear membrane of the oocytes in vacuolated as well as primary yolk granule stages, showed the presence of such 'blebs'

number of nucleoli. Meanwhile, these started lying inner to the nuclear membrane where they finally formed a regular layer. Multiple nucleoli apparently reflect an amplification of genes (Vincent *et al.*, 1969; Vlad,1976; Monaco *et al.*, 1980).

Atresia

Follicular atresia is a degenerative process by which oocytes in various stages of development and differentiation are lost from the ovary (Guraya,1973). Mostly atresia affects the vitellogenic and fully mature yolky eggs. However, atresia is previtellogenic follicles has also been observed (Guraya *et al.*, 1975, 1977).

In teleosts, Hoar (1955, 1957) has distinguished between pre- and post-ovulatory corpora lutea. Guraya (1973) named them as corpora atretica and corpora lutea, respectively. The former involves hypertrophy of the follicular layer and active phagocytosis of yolk and persists longer than the post-ovulatory corpora lutea, which are formed by hypertrophy of the follicle cells after ovulation.

There is considerable variation in the events associated with atresia of vitellogenic follicles in the fish ovary (Guraya, 1986). However, many workers have divided teleost follicular atresia into four consecutive stages according to the original light microscopical description by Bretschneider and Duyvene de Wit (1947) in *Rhodeus amerus*. In the present study, only vitellogenic follicle atresia was noticed and was divided into 4 stages. The main histological features of atretic stages were erosion of zona radiata, liquefaction of yolk from the cortical region centripetally, hypertrophy and phagocytosis of granulosa

cells and finally formation of an irregular mass consisting of granulosa and thecal cells. Granulosa layer has been reported to play active role in ingestion and digestion of yolk during follicular atresia (Guraya *et al.*, 1975, 1977).

In the present study, the proportion of atretic follicles exhibited gradual increase from stage III to stage V ovaries. However, the maximum percentage of atretic follicles was only 2.

It is suggested that atresia is the ultimate outcome of developmental process in the follicle and that all follicles would age and become atretic unless rescued at a critical stage of development by appropriate hormonal stimuli. Several workers have opined that atresia is caused by the lack of proper gonadotropin secretion or due to hypophysectomy (Chester Jones and Ball, 1962; Hoar, 1965; Barr, 1968b; Sundararaj and Goswami, 1968; Guraya, 1973; Saidapur, 1978). In some fish species, the follicular atresia forms a regular feature during the post breeding season, suggesting an adaptation which temporarily suspends breeding activity during unfavourable environmental conditions. Results of the different studies indicate that both hormonal and environmental factors would regulate the initiation of follicular atresia. However, the exact mechanisms by which this process takes place in the oocyte is yet to be known. Some of the recent works indicate that atretic follicles do not have endocrine function (Guraya, 1976a, 1979b, Saidapur, 1978; Nagahama, 1983). The most likely role played by follicular atresia could be restricting the number of eggs shed during the current spawning season. More eggs may, therefore, be released in a favourable season than in an unfavourable one. The term 'favourable' might relate to the abundance of food, since there is evidence that the latter

projecting into the cytoplasm. These 'blebs' are seen to become part of the endoplasmic reticulum (Scharrer and Wurzelmann, 1969a,b; Riehl 1976a, 1978). The formation of these structures are reportedly associated with the transport of nuclear material across the nuclear membrane into the cytoplasm during the early stages of oocyte growth, when excessive amounts of ribosomes are formed and stored for future use during embryonic development (Raven, 1961; Scharrer and Wurzelmann, 1969a; Wischnitzer, 1973; Davidson, 1976).

The lampbrush chromosomes make their appearance during the diplotene stage in different telosts (Braeckevelt and McMillan, 1967; Lehri, 1968; Baumeister, 1973; Raikova, 1976; Wourms, 1976a, Ramadan *et al.*, 1978; Monaco *et al.*, 1980). Because of their extended configuration, lampbrush chromosomes are not readily visible in routine histological preparations, as reported by some workers. In the present material, however, lampbrush chromosomes were found clearly in the vacuolated oocyte stage. They could not be traced in other stages. Bara (1960) showed the presence of lampbrush chromosomes in the oocytes of *Scomber scomber* and their disappearance immediately prior to germinal vesicle breakdown during maturation. Baumiester (1973) also reported the presence of these chromosomes in *Brachydanio rerio.* In *Lepidocephalus thermalis,* Ritakumari and Nair (1979) observed the lampbrush chromosomes in the perinucleolus oocyte stage and their disappearance in the primary yolk globule stage. The lamp-brush chromosomes are identified as importance sites for the synthesis of RNA and protein (Davidson, 1976).

In the present work, the chromatin-nucleolus oocyte contained a single nucleolus. As the oocyte grew further, there was a corresponding increase in the

can affect fecundity (Woodhead, 1960; Bagenal, 1969).

Reproductive cycle

Most teleosts are cyclic breeders and the ovary varies greatly in appearance at different times in the reproductive cycle. Three ovarian types have been classified according to the pattern of oocyte development (Marza, 1938). The synchronous ("Synchronous total") ovary contains oocytes all at the same stage of development. Further replenishment of one stage by an earlier stage does not take place; this type is found in teleosts which spawn only once and then die, such as anadromous *Oncorhynchus* species or catadromous eels. The group synchronous ("Synchronisms par groups") ovary consists of atleast two populations of oocytes at different developmental stages, a fairly synchronous population of larger oocytes and a more heterogenous population of smaller oocytes from which this clutch is recruited. This type is most common among teleosts and may be highly variable among different species. Fishes with this type of ovary such as *Liopsetta obscura* and *Salmo gairdneri* generally spawn once a year and have a relatively short breeding season. On the other hand, *Oryzias latipes*, which has a prolonged breeding season also falls into this category. The asynchronous ("Metachrone") ovary contains oocytes at all stages of development; this type occurs in those species which spawns many times during an extended breeding season. *Fundulus heteroclitus* and *Trachurus trachurus* are two examples for this type of oocyte development.

All the fish species with protracted spawning seasons and multiple spawnings need not necessarily represent the asynchronous oocyte development pattern (de Vlaming, 1983). For example, *Oryzias latipes* is a multiple

spawner, but with four distinct clutches of oocytes, with the advanced clutch being almost synchronous in development. This is characteristic of group synchrony. In a typical asynchronous type of ovary, there occurs continous recruitment into vitellogenesis and the oocytes show no pronounced clutches. This situation is found in *Fundulus heteroclitus*. de Vlaming (1983) remarks that distinguishing between ovaries with multiple clutch group-synchrony and those with typical asynchronous development could become a semantic exercise. One of the most common patterns in group synchronous ovaries is the appearance of three clutches of oocytes just prior to and during the spawning season. The leading clutch is post vitellogenic or in the process of vitellogenesis, a second clutch is in the vacuolated stage (or yolk vesicle stage in other fishes), and there is a third clutch of non-yolky oocytes in the primary growth phase.

In the present work, an attempt was made to determine the type of oocyte development in *S. sihama*, based on the percentage frequency of various oocytes in different maturity stages of ovary. The percentage of primary oocytes was highest in all maturity stages, though their relative volume was low. Vacuolation in the oocytes showed marked rise in stage II ovary, but steadily declined in the next two advanced stages, indicating the absence of recruitment from the primary oocyte stock during mature and ripe conditions. The yolk granule oocytes first appeared in the stage II ovary and increased in stage III. Development of the oocytes from fully yolked condition to hyaline stage, when they were ready for ovulation and spawning, was clearly seen in stage IV ovary. In stage V ovary, vacuolation of the primary oocytes has increased considerably compared to the previous stage. Few oocytes in the primary and secondary yolk granule stages were present. Apparently the yolked oocytes were either developed into the

hyaline stage or become atretic. In the stage V ovary, about 3 percent were hyaline oocytes, which would most likely get resorbed. Percentage of atretic oocytes has increased gradually from stage III to stage V.

With conventional ova-diameter frequency study, it was shown earlier (Chapter IV; Plate VIII) that the ripe ovary contains four batches of ova. Histologically it was found that the most advanced batch consisted by hyaline oocytes, the second batch of yolk granule oocytes, the third batch vacuolated oocytes and the fourth non-yolky oocytes in the primary growth phase. Based on this observation, it may be stated that the oocyte development in *S. sihama* is 'multiple clutch group synchrony', the term being coined by de Vlaming(1983), apparently for a condition between typical group synchrony and extreme asynchrony.

Many workers have expressed the opinion that the conventional ova diameter frequency method alone to identify batches of eggs may be of doubtful value, since histologically recognizable groups of oocytes may not closely correspond to discrete groupings of diameter measurements (Aslanova, 1954; Yamamoto and Yamazaki, 1961; Chigirinsky, 1970; Macer, 1974). Yamamoto and Yamazaki (1961) found it difficult to judge the actual groups of eggs to be spawned at a time, from the ova diameter polygon. The group of largest oocytes in the fully mature ovary of *Carassius auratus* contained oocytes in tertiary yolk globul stage, migratory nucleus stage and pre-maturation stage, which could not be told apart in the usual ova diameter frequency polygon. Unlike this situation, in the present material, it was possible to show histologically that the most advanced group of ova in ova diameter frequency polygon of ripe ovary, consisted only of hyaline oocytes. Macer (1974) stated difficulty in judging

the batch size estimate based on ova diameter frequency polygon in *Trachurus trachurus*. Although he could identify, apart from a clear mode of hyaline oocytes, another group of fully yolked ova, the latter was rarely completely separated from the yolkless ova. In *S. sihama*, the demarkation between the yolked and yolkless oocytes was more clear.

The present histological study, involving the measurement of 10 largest oocytes in the ovaries sectioned during each month showed that the mean diameter increased from July through September, reaching a peak in November and gradually declining to February. This observation agrees well with study of reproductive cycle using gonadosomatic index.

Yolk granule oocytes and hyaline oocytes were relatively more important than the other oocyte stages in the stage III and stage IV ovaries, respectively, as seen from the results of 'relative volume' determination. In other words, it is suggestive of the relative importance of yolk granule oocytes and hyaline oocytes during the phases of active vitellogenesis and spawning, respectively.

In the present material, empty ovarian follicles were infrequent in histological sections. Several workers (Wheeler, 1924; Yamamoto, 1956a; Yamamoto and Yoshioka, 1964; Macer, 1974; Hunter and Goldberg, 1980) have attributed the scarcity of empty follicles in their histological materials to their quick disappearance. In *Oryzias latipes*, Yamamoto and Yoshioka (1964) found that the empty follicles disappeared in 3 days. However, histological evidence of imminent or recent spawning was provided by the presence of hyaline oocytes.

Testis

Testicular structure in teleosts is variable though two basic types, namely 'lobular' and 'tubular' types, can be identified according to the differentiation of the germinal tissue (Billard *et al*.,1982). The 'lobular' type of testis consists of numerous lobules separated from each other by a thin layer of fibrous connective tissue; the arrangement of the lobules varies considerably (Roosen-Runge, 1977). In the 'tubular' type of testis, the tubules are regularly oriented between the external tunica propria (blind end) and a central cavity into which the spermatozoa are released.

Till 1980s, there was no established criteria to distinguish between the two types of testes and the nomenclature used gave rise to a lot of confusion. Lofts and Marshall (1957) and Lofts and Berns (1972) considered it appropriate to call the teleostean male gonad as 'lobular' testes since, unlike the mammalian seminiferous tubule, there is no permanent germinal epithelium in teleost fishes. However, the terms 'lobule' and 'tubule' were used interchangingly without any distinction by several workers (Henderson, 1962; Sanwal and Khanna, 1972; Shrestha and Khanna, 1976, 1978; Dalela *et al*.,1976,1977; Leatherland and Sonstegard, 1978).

Grier *et al*.,(1980) re-examined the testes of four orders of teleosts (Salmoniformes, Perciformes, Cypriniformes and Atheriniformes) and described two types of testes, namely 'unrestricted' testis, in which spermatogonia are distributed all along the entire length of the tubule and 'restricted' testis in which spermatogonia are totally restricted to the distal terminal part of the tubule. Along teleosts, the 'restricted' testis is almost exclusively found in Atheriniform fishes, which fall into the category of 'tubular' testis according to the classification by Billard *et al*.(1982).

In the present study, the structure of the testis in *S. Sihama* was found to be the 'lobular' type following the description of Billard *et al.* (1982). The presence of germinal cysts all along the inner lobular wall indicates that the testis is of the 'unrestricted' type, as described by Grier *et al.* (1980).

Since long, it has been established that the testis of teleosts produce androgens (Pickford and Atz, 1957), but the site of androgen production within the testis has been a matter of controversy (Courrier, 1921, 1925; Champy, 1923a,b; Van Oordt, 1924b,c; Craig-Bennett, 1931). Steroidogenic function has been attributed to the interstitial and lobular compartments of the testis, which are considered homologous with the Leydig cells and Sertoli cells of mammals. The interstitium between the lobules consists of interstitial cells, fibroblasts, and blood and lymph vessels. The lobular compartment of the teleost testis consists of germ cells and distinct somatic cells lining the periphery of the lobule. The Sertoli cells may either be secretory or non-secretory in function. The Leydig cells on the other hand, are found to have features of steroid-producing cells. Histochemically 3 β-hydroxy- Δ^5-steroid dehydrogenase (3 β-HSD). an enzyme known to be involved in steroid hormone synthesis, has been demonstrated in the interstitial cells of the testis of a number of teleosts (Nagahama *et al.*, 1982).

In the present work, it was not possible to locate Sertoli cells with the limitation of light microscopy. But Leydig cells were seen in the interstital space between lobules in mature testis. In *Mugil cephalus*, Grier (1981) reported that Leydig cells were difficult to find in the interstitium of fish undergoing recrudescence, but more reasonably common within the intersitium

of fish at the nadir of the reproductive cycle. In most of the sections observed in the present study, the interlobular somatic tissue was so closely packed that it was difficult to differentiate each cell type.

In teleosts, sperm ducts (vasa diferentia) are formed independantly by somatic cells derived from the coelomic wall; they are in no way a part of the nephric duct or Wolfian duct (Nagahama, 1983). In *S. sihama* the vas deferens was found situated in the dorsal surface of the testis initially, and as development preceeded, came to occupy the inner lateral side of the testis. The vas deferens gives rise to the primary vasa efferentia that extend dorsoventrally, giving away smaller branches (secondary vasa efferentia) throughout the body of the testis. They inturn become continuous with the lumens of germ-cell cysts, so that the germ cells come to line the walls of the lobules. The efferent system in *S. sihama* was found to be more elaborate than the same reported in Atheriniform fishes (Billard *et al.*, 1982), which have a 'restricted' type of testis. The branching of the vasa efferentia and the arrangement of lobules in the present material also seems to be of the 'radial type', described in several teleosts (*Perca flavescens* : Turner, 1919; *Oncorhynchus nerka:* Weisel, 1943; *Esox lucius:* Lofts and Marshall, 1957; *Galaxias maculatus:* Henderson, 1962; *Tilapia nilotica:* Latif and Saady, 1973), in which lobules converge radially on the main sperm duct. An opposite situation exists in *Amphipnous cuchia* (Rastogi, 1968) and *Tandanus tandanus* (Davis, 1977), in which the lobules run lengthwise with lateral communications becoming evident during the advanced stages of maturity.

Spermatogenesis

In teleosts, development of germ cells take place within the cysts formed by Sertoli cells. Turner (1919) used the term "germ cells" for the predecessors of spermatogonia, while Kristoffersson and Pekkarinen (1975) named them "primary germ cells". Some workers have identified different types of spermatogonia in teleost ovary. Michibata (1975) differentiated three types of spermatogonia, namely "spermatogonia A", "spermatogonia undifferentiated" and "spermatogonia B" based on the size difference, while Remacle *et al.*, (1977) recognized two types of spermatogonia, "primary spermatogonia" and "secondary spermatogonia". The primordial germ cells were called by the term "stem speratogonia" by Billard (1979). He homologued them with the "Type A speramogonia" of mammals, and believed that a second type ("Type B spermatogonia") differentiate from them. Brusle and Brusle (1978a, b) have introduced the term "primordial germ cells", which were considered to be bipotential, being present both in males and females, during the embryonic development of the gonad. In mullets, they identified only one type of spermatogonium followed by primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa.

In the present study, in *S. sihama* 6 spermatogenic stages were differentiated, namely primordial germ cell, spermatogonium, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoan/sperm. Two types of spermatogonia were apparent in the immature testis. Initially the speratogonia were smaller than the primordial germ cells with the nucleus staining lightly. As they underwent a period of growth, they became the largest cells of

all the spermatogenic stages. The nucleus stained more intensely at this stage. These two types of spermatogonia observed in the testis of may probably be corresponding to the 'primary spermatogonia' and 'secondary spermatogonia' identified by Remacle *et al.* (1977). Each spermatogonium undergoes mitotic division to form cytsts of germ cells.

Turner (1919) suggested that as many as six divisions occur in order to give rise to the number of spermatocytes in a cyst. Turner (1919), Han (1927) and Weisel (1943) reported that each cyst has a fine membraneous caspule composed of flattened duct epithelial cells. In the testis of *Gerres*, Cyrus and Blaber (1984) have also observed a nest membrane for the nests of spermatocytes. However, a well delineated cyst capsule does not appear to occur in *S. sihama*. A similar observation was made in mullets by Stenger (1959).

As in other teleosts, there was a gradual decrease in the size of cell from the spermatogonium to the spermatozoan, which may indicate that the growth phase of the cell almost completes at the spermatogonial stage itself. Staining intensity gradually increased from the primordial germ cells to spermatids, showing greater condensation of the chromatin material with the progress in spermatogenic development. It has been reported that the secondary spermatocytes are found in less numbers in the testis, since they rapidly get transformed to spermatids (Nagahama, 1983). In *S. sihama* too, a similar observation was made.

The type of spermatogenesis in *S. sihama* was found to be 'cystic' (Mathews and Marshall, 1956; Lofts, 1968), with synchronous development of cysts in the same phase of development were few inside the lobule. In *Liza parsia*, Joseph (1987) has found an identical situation. In *S. sihama* all the

spermatogenic stages, including spermatozoa were seen in the advanced maturing stage. Spermatogenic activity was most intense in stage III testis. Central lobules of the testes appeared to become distended with spermatozoa earlier than the peripheral lobules, as in other teleosts (Turner, 1919; Craig-Bennett, 1931). Since the present work was limited to light microscopical observations, spermiogenesis could not be studied. Neverthless, one of the morphological changes associated with spermiogenesis, namely the shrinkage of nucleus, has been noticed in the present material. The mean diameter of nucleus descreased from 1.57 µm in the spermatid to 1.2 µm in the spermatozoan.

Clusters of newly formed sperms appeared in section as fanshaped, or as Turner (1919) described them, "parachutes". It is possible that the 'parachute' formation is the result of adhesion of sperm tails. In the stage IV testis, spermatozoa occurred as dense, unorganised masses in the lobules and the vas deferens.

On the basis of the observations made in the present study, the process of testicular maturation in *Sillago sihama* can be summed up as follows:

The testicular differentiation begins with the formation of isolated lightly staining groups of primordial germ cells, which undergo cell division and organise themselves into distinct lobules. Each primordial germ cell transforms into a spermatogonium, which after a period of growth, multiplies and give rise to cysts of primary spermatocytes. Each primary spermatocyte in the cyst undergoes first meiotic division and gives rise to two secondary spermatocytes. Each secondary spermatocyte undergoes second meiotic division and gives rise to two spermatids. The spermatids get transformed into spermatozoa with the formation of tail. The spermatozoa, when they are initially formed, assume a

TABLE 37. Biochemical Composition of Blood plasma of male *S. sihama* during different stages of maturity

Stages	Glucose (mg/100 ml)	Protein (g/100 ml)	Lipid (g/100 ml)	Cholesterol (mg/100 ml)
I	275.61 ±15.65	9.00 ±0.25	4.50 ±0.20	300.50 ±25.50
II	405.68 ±25.75	9.30 ±0.30	4.00 ±0.10	290.35 ±15.00
III	400.50 ±25.00	6.00 ±0.10	4.00 ±0.2	190.45 ±10.00
IV	433.40 ±15.65	2.80 ±0.10	3.50 ±0.10	142.05 ±15.65
V	200.50 ±25.00	4.50 ±0.20	4.50 ±0.20	276.36 ±25.00

CHAPTER VI

BIOCHEMICAL CHANGES DURING MATURATION AND SPAWNING

Biochemical changes during gonadal maturation of a number of temperate and sub-tropical teleost fishes have been investigated. *Clupea harengus* was the subject of a number of biochemical studies (Milroy, 1908; Bruce, 1924; Channon and Saby, 1932; Lovern and Wood, 1937). Fat content variations in several species have been studied in relation to maturation. Some of the noteworthy works on this aspect include, Lofts and Marshall (1959) in *Esox lucius*, Wilson (1939) in flounders, Ackman and Eaton (1971) in mackeral, Deng *et al.* (1976) and Dindo and MacGregor (1981) in *Mugil cephalus*.

Variations in protein content were investigated in *Salmo gairdneri* (Gras *et al.*, 1967a), *Gadus morhua* (Ipatov, 1970) and *Esox lucius* (Medford and Mackay, 1978). Blood sugar content was observed to vary in accordance with maturity stages in *Opsanus tau* (Schub and Nace, 1961) and *Catstomus commersonii* and *Esox lucius* (Mackay and Beatty,1968b). All these works have shown that the reproduction in fish is an energy demanding activity and brings about drastic changes in the biochemical composition of the fish.

A perusal of the literature on biochemical variations of Indian fishes indicates that most of the works have been carried out in freshwater species. such as *Heteropneustes fossilis*, *Clarias batrachus*, *Channa punctatus*

and *Cirrhinus mrigala*. In these investigations, biochemical anlyses of various tissues like muscle, liver, gonads and blood of the fishes have been done with a view to understanding the changes accompanying sexual maturation. Seasonal variations in body composition of *Heteropneustes fossilis* were studied by Chaturvedi *et al.* (1976) and Pandey *et al.* (1976). Fat content variations in *Clarias batrachus* was studied by Yagano Bano and Hameed (1979). Changes in blood and ovarian cholesterol content of *Ophicephalus punctatus* (Siddiqui, 1968), *Anabas testudineus* (Sen and Bhattacharya, 1981) and *Cirrhinus mrigala* (Singh and Singh, 1984) have been investigated. Variations in protein content of *Lebeo gonius* during maturation were studied by Jain and Singh (1981). Blood glucose and glycogen level changes in *Channa punctatus* (Khanna and Singh, 1971) and *Anabas testudineus* (Dasgupta and Sircar, 1986) have been reported.

Only few Indian marine teleosts have been the subject of similar investigations. Some of the noteworthy works include, studies of fat and water content variations in *Pseudosciaena aneus* and *Johnius carutta* (Rao, 1967), *Sardinella longiceps* (Sen and Challuvaiah, 1968) and proximate composition of *Ambassia gymnocephalus* (Vijayakumaran, 1979). Recently Joseph (1987) studied biochemical variations in *Mugil cephalus* and *Liza parsia* accompanying sexual maturation.

But for a few figures of inorganic constituents and vitamins (Love, 1970), there is no work on the biochemical composition of sillaginid fishes in relation to maturation of gonads in Indian waters or elswhere. As the information on the biochemistry of the different body constituents during gametogenesis of marine fishes in India is scarce and since such information

on the sillaginids is not available, the present study on the variations of moisture, total carbohydrates, protein, glycogen, glucose, lipid and cholesterol in liver, muscle, gonads and blood plasma of both female and male *Sillago sihama* in relation to maturity stages, was taken up.

OBSERVATIONS

The data on estimated moisture content, total carbohydrates, protein, glycogen, lipid and cholesterol in muscle, liver and gonads of both sexes of *S.sihama* during different stages of maturity along with the corresponding standard deviations are given in Tables from 28 to 35 and depicted in plates from XXIII to XXXIV. To obviate the influence of moisture content on the estimation of these parameters, analyses of the various tissues were carried out on dry weight basis besides th estimation on fresh tissue.

Female fish

Muscle:

The data on the biochemical composition of fresh muscle during different stages of maturity are given in the Table 28. The values estimated on dry weight basis are given in the Table 34.

Moisture:

The range of moisture content in the muscle was from 75.56 to 77.51%. After a slight increase from Stage I to Stage II, moisture content showed decreasing values in the subsequent stages (Plate XXIII).

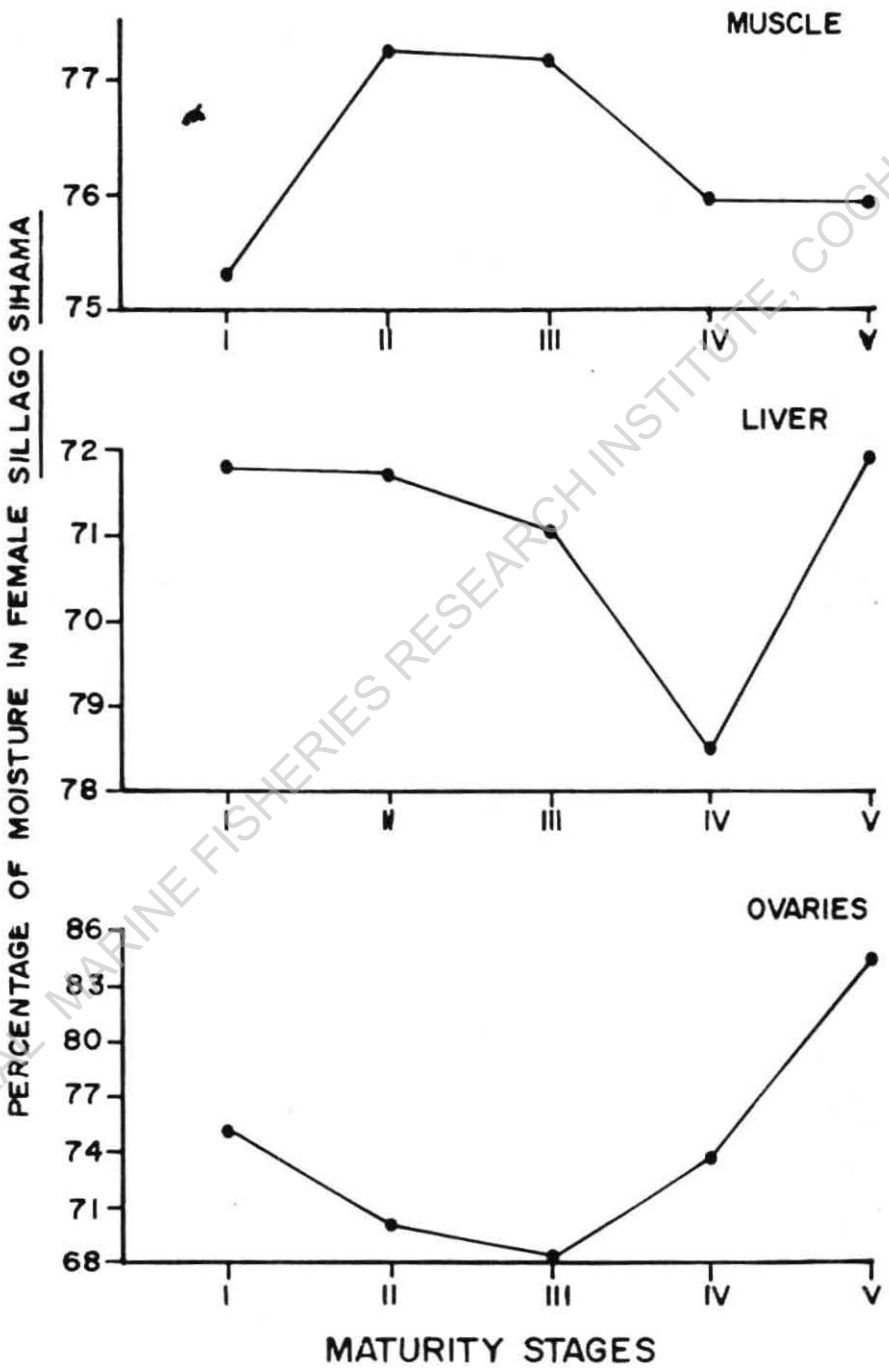
TABLE - 28. Biochemical composition of Fresh Muscle tissue of female *S. sihama* during different stages of maturity

Stages	Moisture %	Total Carbohydrates %	Protein %	Glycogen %	Lipid %	Cholesterol %
I	75.56 ±0.21	0.58 ±0.07	16.21 ±0.87	0.0060 ±0.001	2.08 ±0.19	0.143 ±0.017
II	77.51 ±0.60	0.28 ±0.02	17.39 ±0.88	0.0056 ±0.0010	1.83 ±0.43	0.105 ±0.006
III	77.27 ±0.07	0.39 ±0.02	10.76 ±1.25	0.0064 ±0.0020	1.51 ±0.10	0.134 ±0.007
IV	75.85 ±0.11	0.47 ±0.01	6.77 ±1.91	0.0022 ±0.0020	1.19 ±0.10	0.134 ±0.012
V	75.93 ±0.11	0.50 ±0.01	8.12 ±1.25	0.0063 ±0.0020	1.85 ±0.10	0.142 ±0.007

PLATE XXIII.

Variation of lipid content in the muscle, liver and ovaries of *S. sihama* during different maturity stages.

PLATE XXIII



Total carbohydrates:

The total carbohydrate content of the muscle varied from 0.28 to 0.58%. It was highest in the immature stage and after a sharp decline in Stage II, steadily increased till stage V (Plate XXIV). On dry weight basis, the carbohydrate content showed similar trend in variation, with range of 1.24-2.38%.

Protein:

Protein content in the muscle ranged between 6.77 and 17.39%. After an increase in stage II, the protein content decreased till stage IV and then registered about 2% increase in stage V (Plate XXV). On dry weight basis a similar trend was observed, with a range of 28.01-78.22%.

Glycogen:

The range of glycogen content in the muscle of female fish was from 0.0022% to 0.0064%. There was an increasing tendency of this constituent from stage I to stage III, a decline in stage IV and a further increase in stage V (Plate XXVI). Almost a similar pattern of variation was noticed on dry weight basis, with a range of 0.0091-0.028%.

Lipid:

Lipid content exhibited a steady decrease from 2.08% in stage I to 1.19% in stage IV and a slight increase to 1.85% in stage V (Plate XXVII). On dry weight basis the range of variation in lipid content was 4.93-8.37%, with a similar trend to that of the fresh tissue.

Cholesterol:

Cholesterol content in the muscle varied between 0.104 and 0.143%.

PLATE XXIV

Variation of total carbohydrate content in the muscle, liver and ovaries of *S. sihama* during different maturity stages.

PLATE XXIV

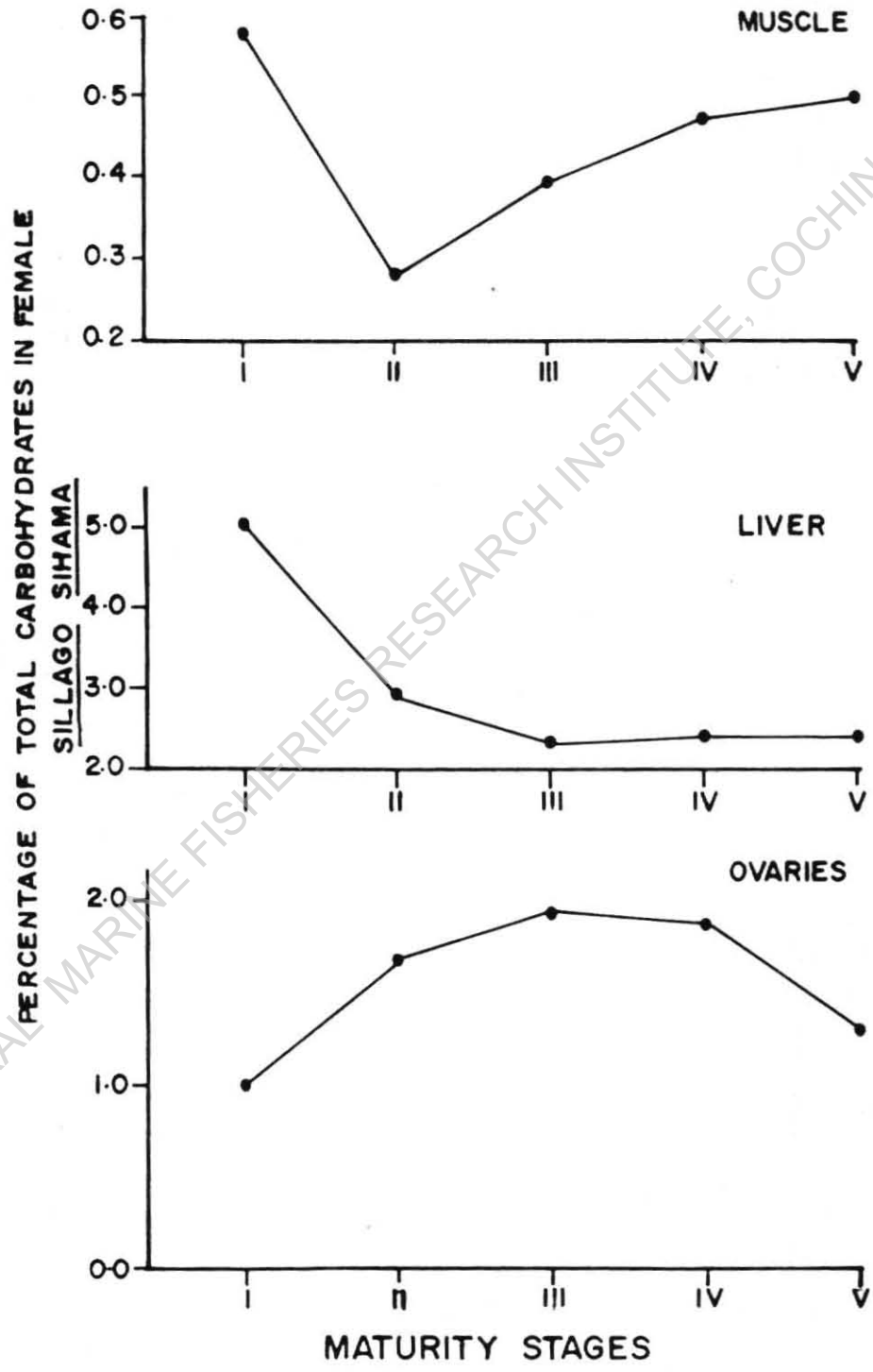


PLATE XXV.

Variation of protein content in the muscle, liver and ovaries of *S. sihama* during different maturity stages.

PLATE XXV

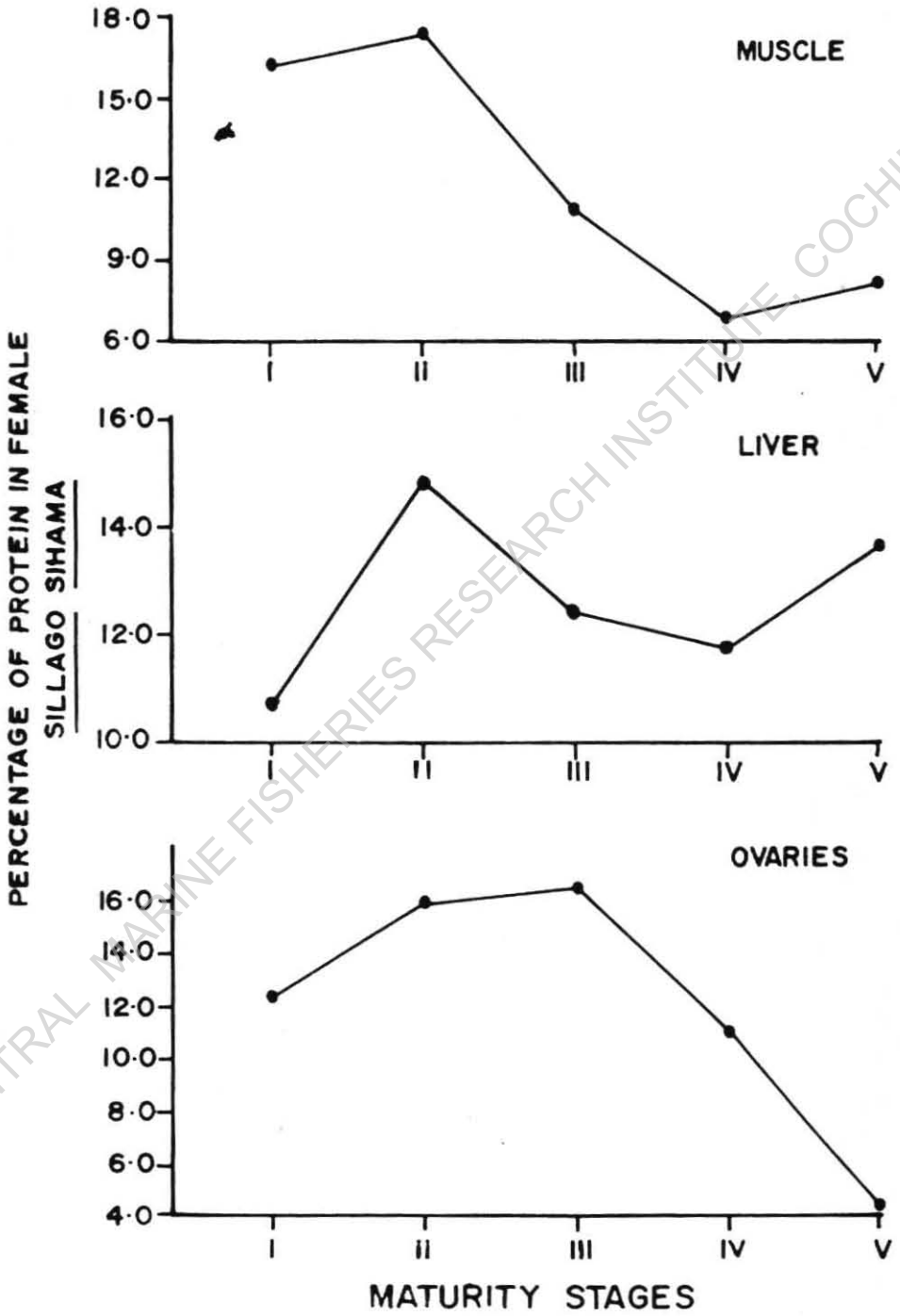


PLATE XXVI.

Variation of glycogen content in the muscle, liver and ovaries of *S. sihama* during different maturity stages.

PLATE XXVI

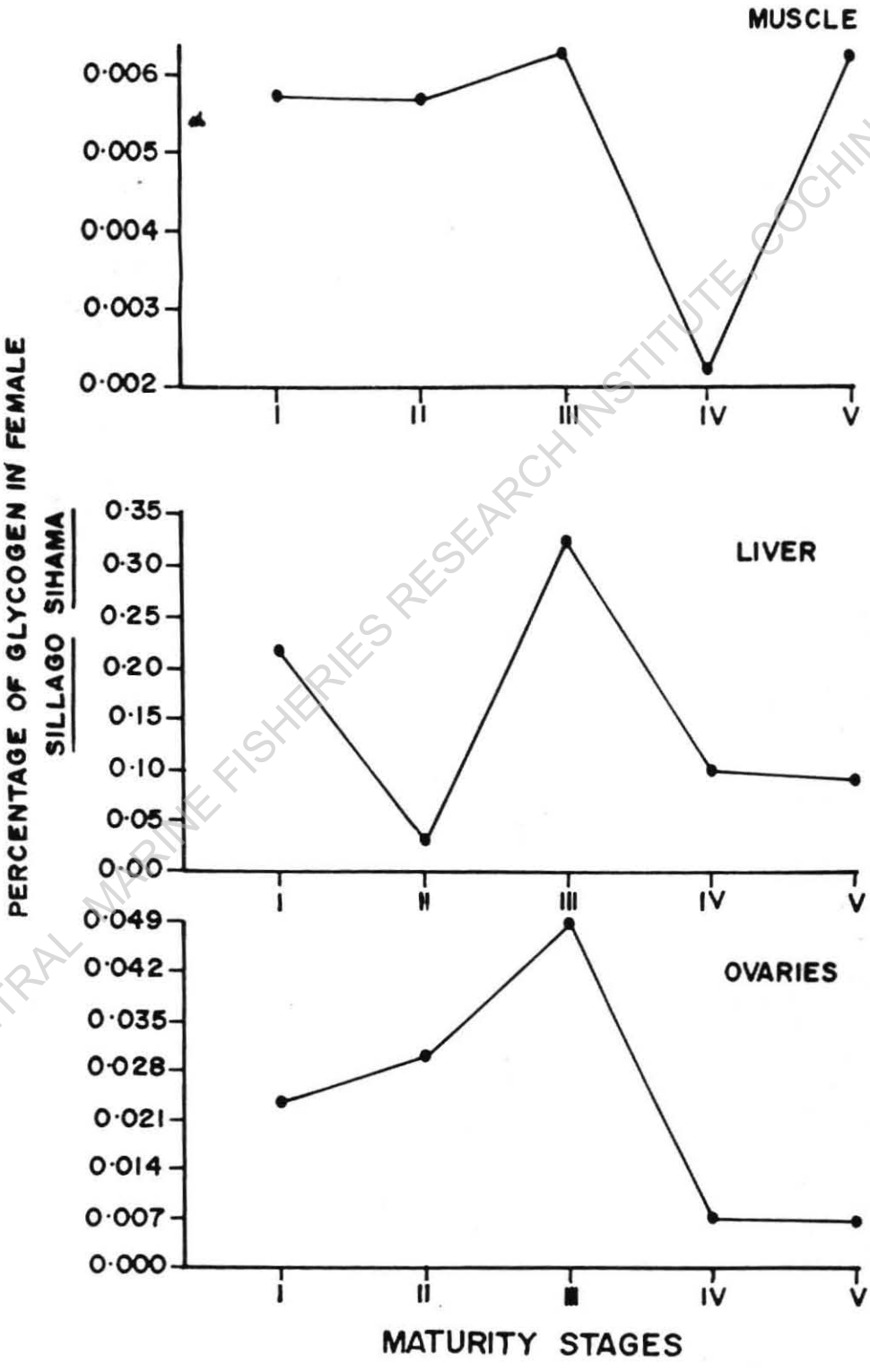
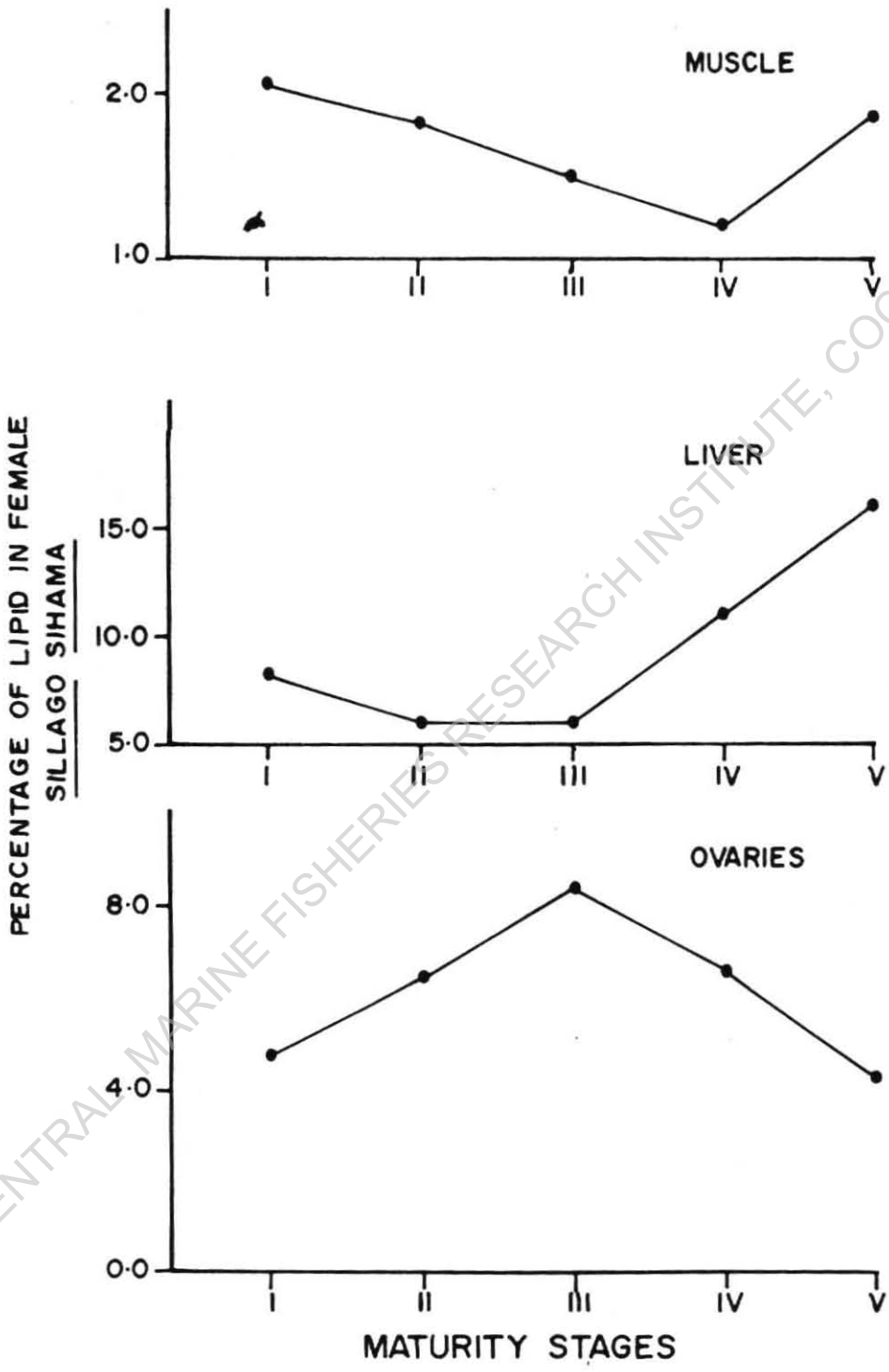


PLATE XXVII.

Variation of lipid content in the muscle, liver and ovaries of *S. sihama* during different maturity stages.

PLATE XXVII



There was a decline from stage I to stage II and then a steady increase till stage V (Plate XXVIII). On dry weight basis the trend in variation was similar, with a range of 0.477-0.632%.

Liver:

The data on the biochemical composition of fresh liver during different stages of maturity are given in the Table 29 and the values estimated on dry weight basis are given in the Table 34.

Moisture:

The range of moisture content in the liver was 68.46-71.93%. The moisture content decreased steadily from stage I to IV and registered an increase by about 4% in stage V (Plate XXIII).

Total carbohydrates:

Total carbohydrate content in the liver varied from 2.3 to 5.04%. Maximum value was recorded in stage I and after a sharp decline in Stage II, it remained low in the subsequent stages (Plate XXIV). A similar trend in variation was observed on dry weight basis with a range of 9.89-17.93%.

Protein:

The protein content in the liver during different stages of maturity varied between 10.72 and 15%. There was a marked increase from Stage I to stage II (Plate XXV), followed by steady decrease till stage IV and a further increase in stage V. The same pattern of variation was seen on dry weight basis also, with a range of 38.22-51.78%..

PLATE XXVIII.

Variation of cholesterol content in the muscle, liver and ovaries of *S. sihama* during different maturity stages.

PLATE XXVIII

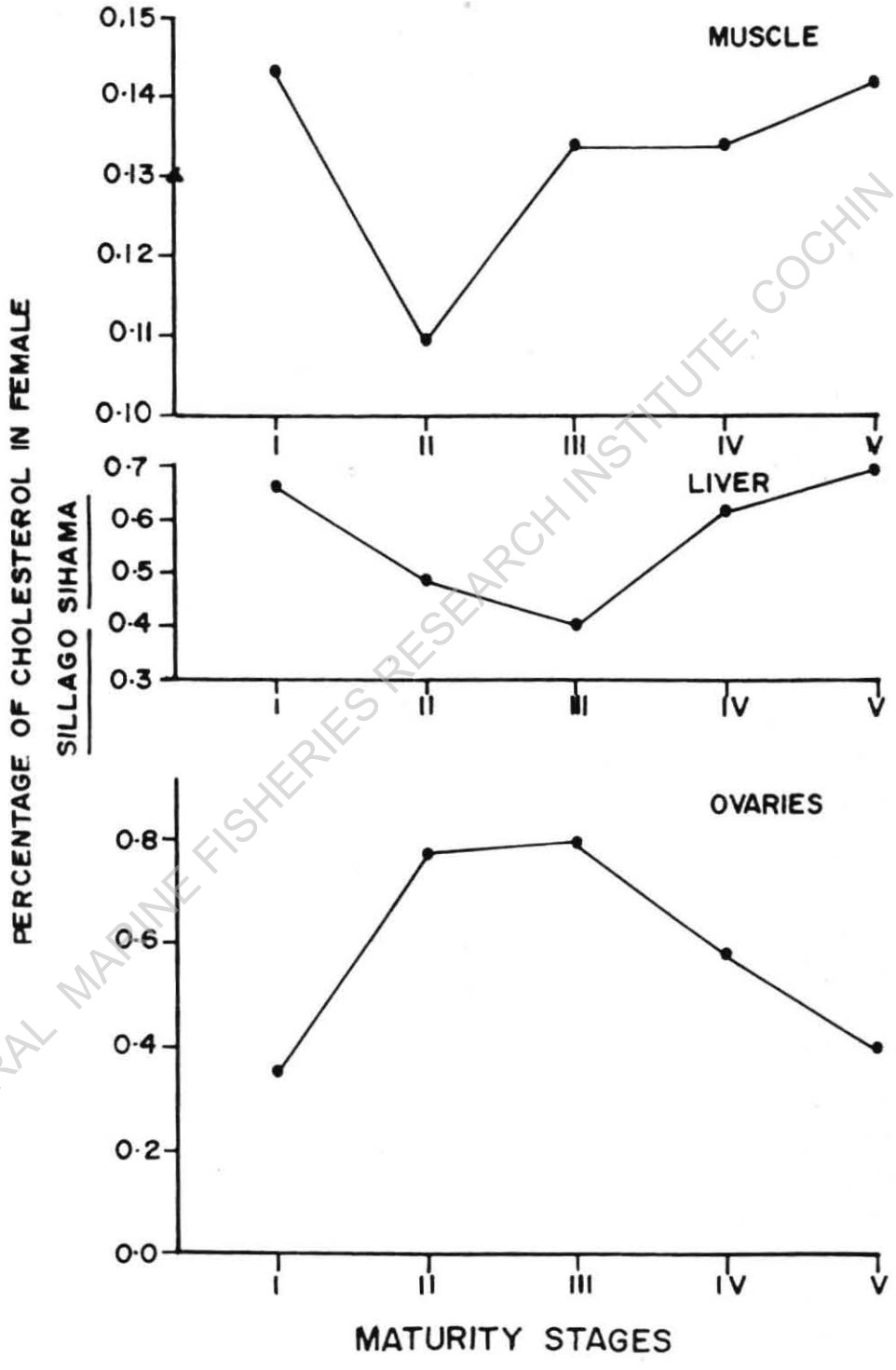


TABLE 29. Biochemical composition of fresh liver tissue of female *S. sihama* during different stages of maturity

Stages	Moisture %	Total Carbohydrates %	Protein %	Glycogen %	Lipid %	Cholesterol %
I	71.78 ±2.59	5.04 ±0.07	10.72 ±0.96	0.220 ±0.010	8.12 ±0.48	0.668 ±0.060
II	71.75 ±1.94	2.90 ±1.31	15.00 ±0.10	0.0302 ±0.001	6.00 ±0.10	0.486 ±0.115
III	71.05 ±0.70	2.30 ±0.21	12.48 ±0.10	0.327 ±0.020	5.08 ±0.37	0.400 ±0.077
IV	68.46 ±4.91	2.39 ±0.20	11.84 ±1.34	0.099 ±.010	11.06 ±0.20	0.619 ±0.100
V	71.93 ±2.60	2.40 ±0.20	13.71 ±0.90	0.090 ±0.010	16.06 ±0.20	0.690 ±0.060

Glycogen:

The range of glycogen content in the liver was 0.03-0.327%. The glycogen content decreased sharply in stage II from stage I and increased to the maximum value in stage III and declined in the subsequent stages (Plate XXVI). On dry weight basis too, the trend in variation was similar, with a range of 0.11-1.1%.

Lipid:

Lipid content in the liver of female fish varied from 5.98 to 16.06%. Unlike protein, lipid content in the liver exhibited a steady decline from stage I to III and marked increase in stages IV and V (Plate XXVII). On dry weight basis, lipid content decreased from 28.77% in stage I to 21.5% in stage III and increased to the maximum value of 50.92% in stage V.

Cholesterol:

Variation of cholesterol content in the liver was similar to that of lipid content. It decreased from 0.668% in stage I to 0.4% in stage III and increased to the maximum level of 0.69% in stage V(Plate XXVIII). On dry weight basis, the range of variation in the Cholesterol content was 1.38-2.24%, the trend being similar to that on the fresh weight basis.

Ovaries:

Biochemical changes in the ovaries were more conspicuous than that in the muscle and liver. The data on the biochemical composition of fresh ovaries are given in the Table 30 and the values estimated on dry weight basis are given in the Table 34.

TABLE 30. Biochemical composition of fresh ovaries of *S. sihama* during different stages of maturity

Stages	Moisture %	Total Carbohydrates %	Protein %	Glycogen %	Lipid %	Cholesterol %
I	75.33 ±0.38	1.01 ±0.38	12.44 ±0.67	0.024 ±0.010	4.63 ±0.82	0.347 ±0.020
II	70.16 ±1.76	1.68 ±0.00	16.11 ±0.46	0.030 ±0.010	6.44 ±0.27	0.773 ±0.043
III	68.39 ±3.38	1.95 ±0.22	16.57 ±0.68	0.049 ±0.010	8.33 ±0.85	0.786 ±0.590
IV	73.83 ±1.76	1.88 ±0.80	11.24 ±0.10	0.007 ±0.000	6.48 ±0.10	0.578 ±0.025
V	84.54 ±2.00	1.31 ±0.22	4.48 ±0.10	0.007 ±0.000	4.17 ±0.10	0.398 ±0.025

Moisture:

The moisture content of the ovaries varied from 68.39 to 84.54%. It decreased steadily from 75.33% in Stage I to 68.39% in stage III and increased significantly to 84.54% in stage IV (Plate XXIII).

Total carbohydrates:

The range of total carbohydrate content in ovaries was 1.01-1.95%. There was a steady increase from stage I to stage III and slight decrease till stage V (Plate XXIV). On dry weight basis, the trend in variation was similar, with a range of 3.65-6.58%.

Protein:

Protein content in the ovaries varied between 4.48 and 16.57%. Variation in protein content during different stages of maturity followed a similar pattern as that of the carbohydrate content, though it was more marked (Plate XXV). On dry weight basis, protein content increased from 50.26% in stage I to 55.93% in stage III and declined to 37.53% in stage IV and 28.98% in stage V.

Glycogen:

The range of glycogen content in the ovaries was 0.007-0.49%. Glycogen content increased from 0.024% in stage I to 0.049% in stage III and decreased to 0.007% in stage V (Plate XXVI).

Lipid:

Lipid content in the ovaries showed a steady increase from 4.63% in stage I to 8.35% in stage III and decreased to 6.48% in stage IV and 4.17% in

stage V (Plate XXVII). On the dry weight basis, the trend in variation in the lipid content showed perceptible difference from fresh tissue between stage III and V. On dry weight basis, the lipid content remained almost same in stages III and IV and there was an increase by about 2% in stage V.

Cholesterol:

Cholesterol content in the ovaries varied from lowest value of 0.347% in stage I to the highest value of 0.786% in stage III. The pattern of variation in cholesterol content was similar to those in total carbohydrates, protein and glycogen, with an increasing trend form stage I to stage III and decreasing trend from stage III to V (Plate XXVIII). On dry weight basis, the range was 1.3-2.61%.

Blood plasma

Data on the biochemical composition of blood plasma of female *S. sihama* during different stages of maturity are given in the Table 36 and depicted in the Plate XXXV.

Glucose content was found to vary from 17.602 to 349.31 mg/100 ml. It increased steadily from stage I to stage III, declined sharply in stage IV and further increased in stage V. Protein content of blood plasma remained almost same in stages I and II, increased in stage III and declined till stage V. The range of protein content was 2-5.13g/100ml. Lipid content showed similar trend in variation with a range of 0.8-2.6 g/100 ml. Cholesterol content in the plasma increased from 180.53 mg/100 ml in stage I to 220.47 mg/100 ml in stage III and decreased to the lowest value of 100.6 mg/100 ml in stage V.

Male fish

Muscle

The data on the biochemical composition of fresh muscle during different stages of maturity are given in the Table 31 and the values estimated on dry weight basis are given in the Table 34.

Moisture:

The percentage of moisture content in the muscle varied only slightly, with relatively higher values in stages III, IV and V (Plate XXIX). The range was 76.4-78.7%.

Total carbohydrates:

The range of total carbohydrates in the muscle was 0.41-0.76%. There was decreasing trend till stage IV from stage I and a further increase in stage V (Plate XXX). On dry weight basis, the trend in variation was similar with a range of 1.74-3.23%.

Protein:

Protein content in the muscle varied between 14.08 and 16.19%. It decreased from stage I to stage III and increased in the subsequent stages (Plate XXXI). On dry weight basis, protein content decreased from 63.34% in stage I to 58.87% in stage III and increased steadily to 71.82% in Stage V.

Glycogen:

The range of glycogen content in the muscle during different stages of maturity was 0.0021-0.0057%. Glycogen content increased from stage I to state III, decreased in stage IV and slightly increased in stage V (Plate XXXII).

TABLE 31. Biochemical composition of fresh muscle tissue of male *S. sihama* during different stages of maturity

Stages	Moisture %	Total Carbohydrates %	Protein %	Glycogen %	Lipid %	Cholesterol %
I	76.98 ±1.27	0.60 ±0.23	15.15 ±0.20	0.0021 ±0.000	1.32 ±0.10	0.160 ±0.025
II	76.40 ±0.13	0.43 ±0.10	14.49 ±0.18	0.0057 ±0.001	0.43 ±0.08	0.142 ±0.015
III	78.05 ±0.22	0.43 ±0.10	14.08 ±0.01	0.0056 ±0.000	1.03 ±0.10	0.117 ±0.025
IV	78.00 ±0.10	0.41 ±0.10	15.18 ±1.00	0.003 ±0.010	1.50 ±0.57	0.027 ±0.01
V	78.70 ±0.20	0.76 ±0.23	16.19 ±1.43	0.0040 ±0.001	1.90 ±0.5	0.060 ±0.01

PLATE XXIX

Variation of moisture content in the muscle, liver and testes of *S. sihama* during different maturity stages.

PLATE XXIX

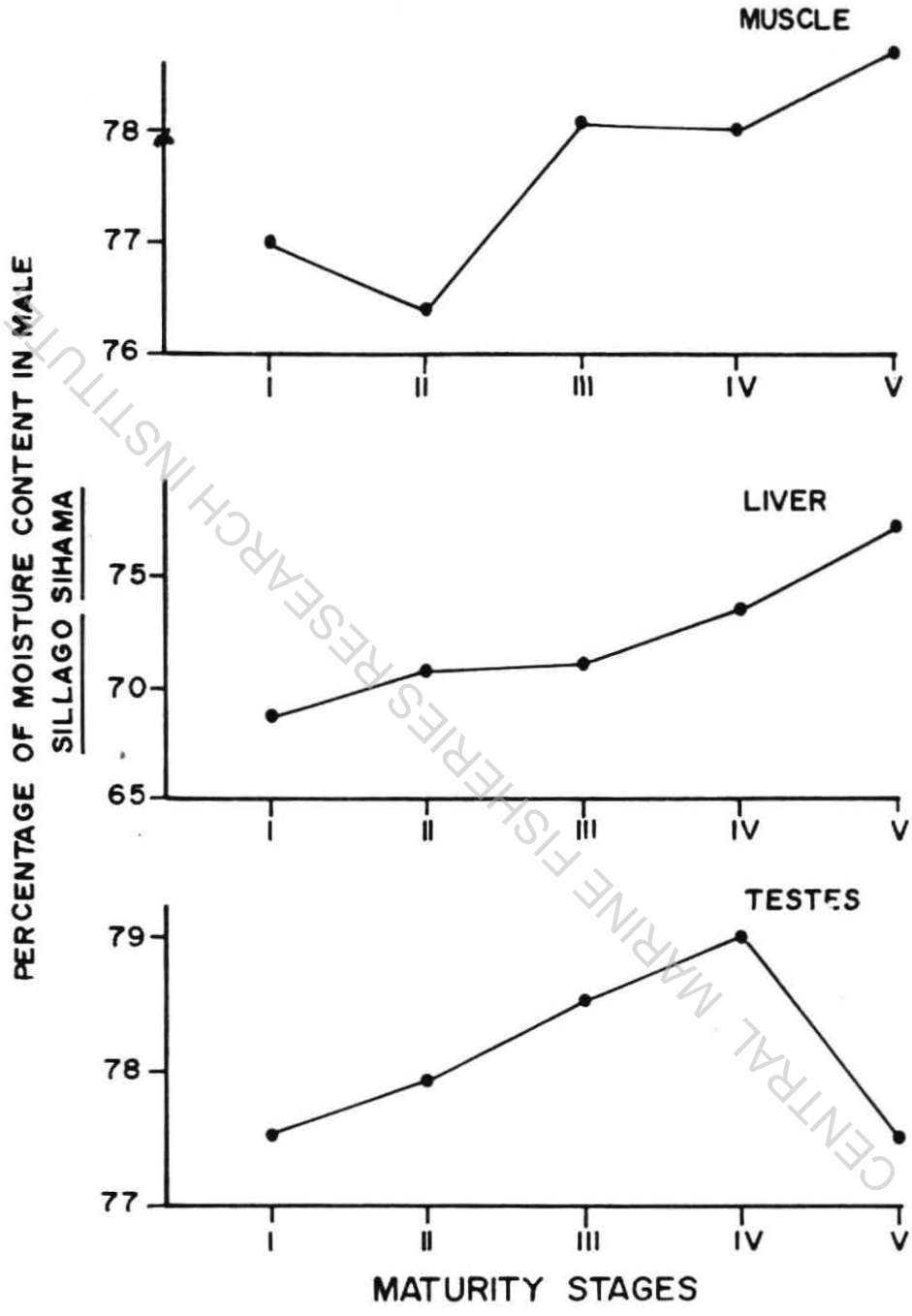


PLATE XXX.

Variation of total carbohydrate content in the muscle, liver and testes of *S. sihama* during different maturity stages.

PLATE XXX

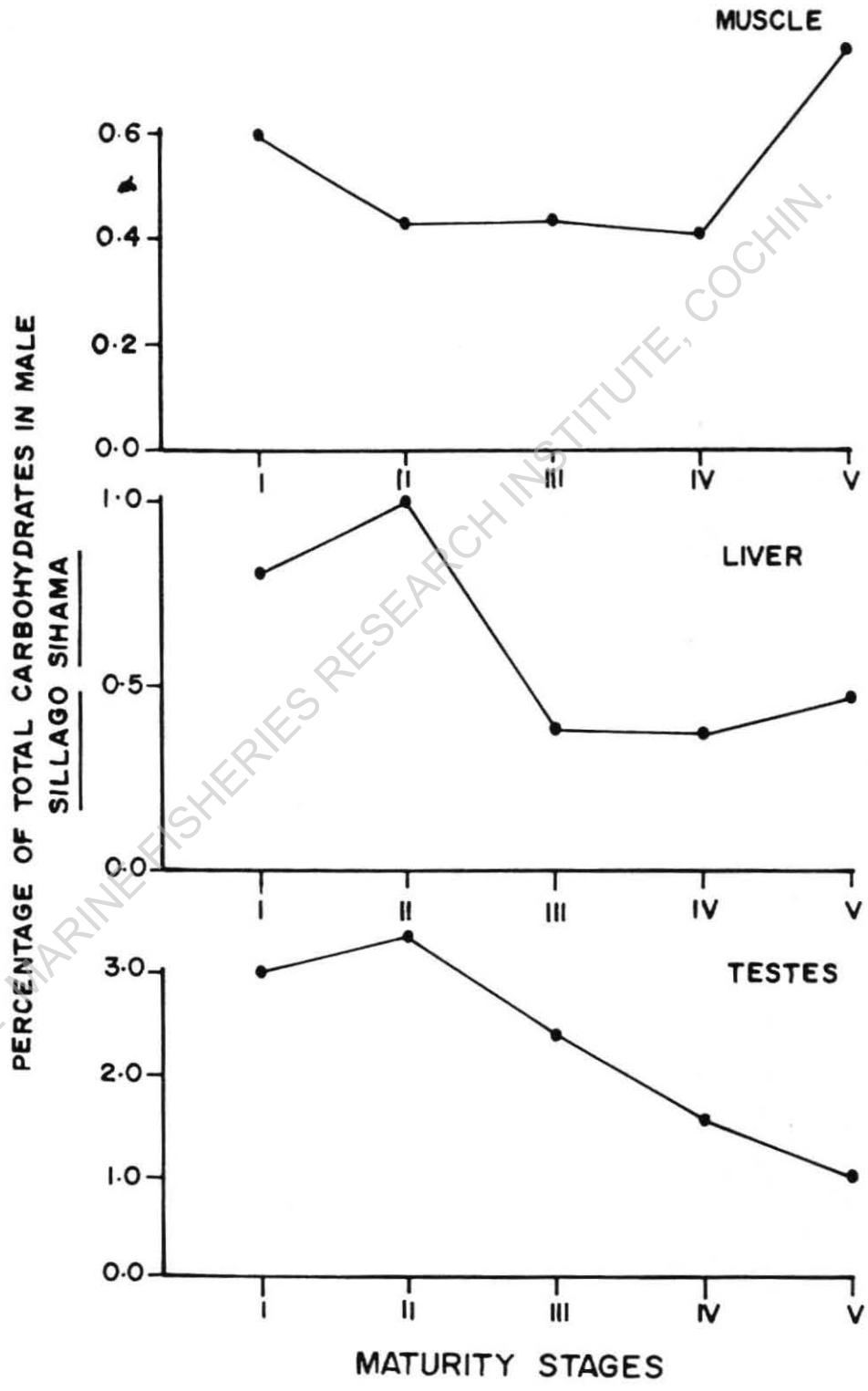


PLATE XXXI

Variation of protein content in the muscle, liver and testes of *S. sihama* during different maturity stages.

PLATE XXXI

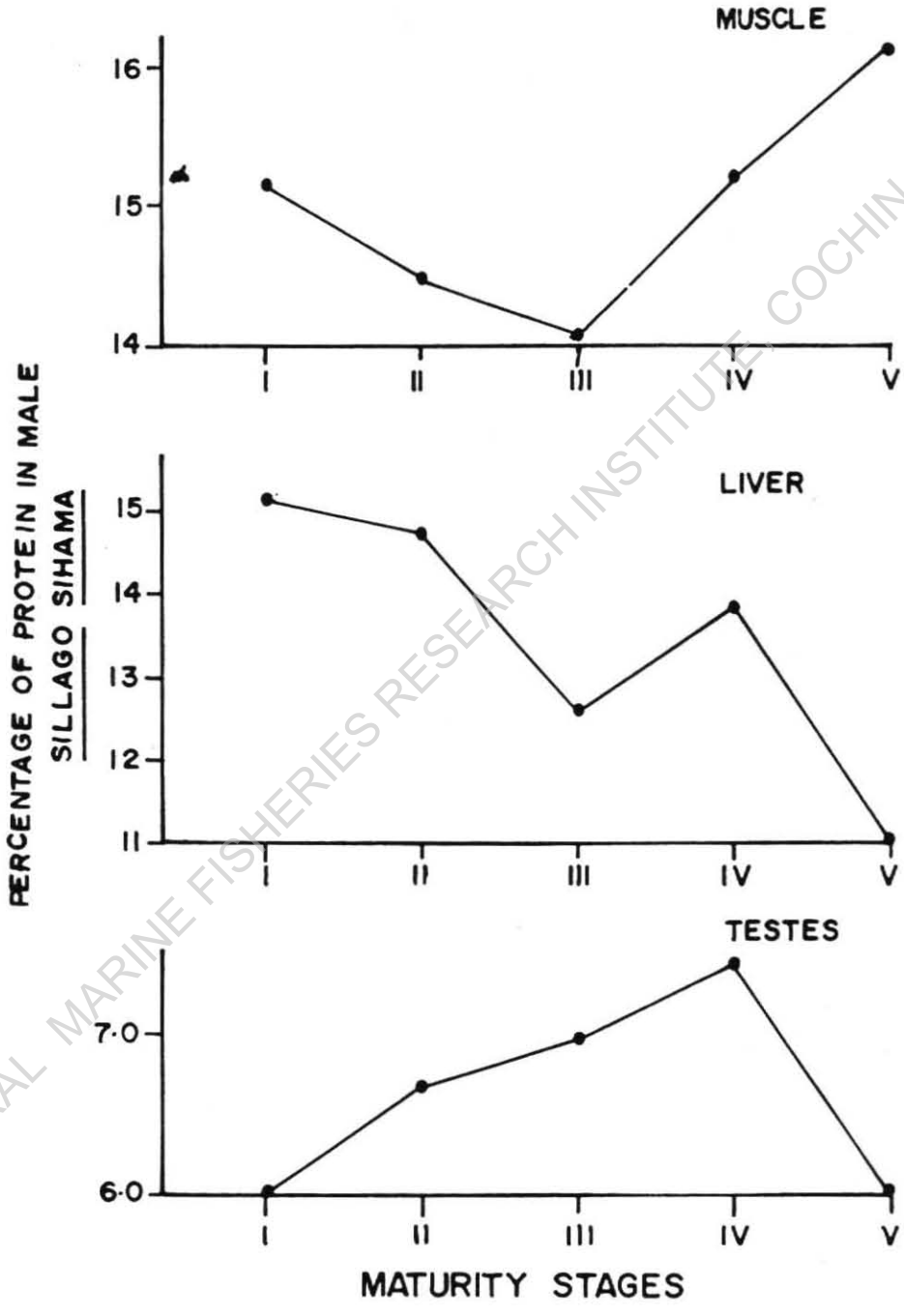
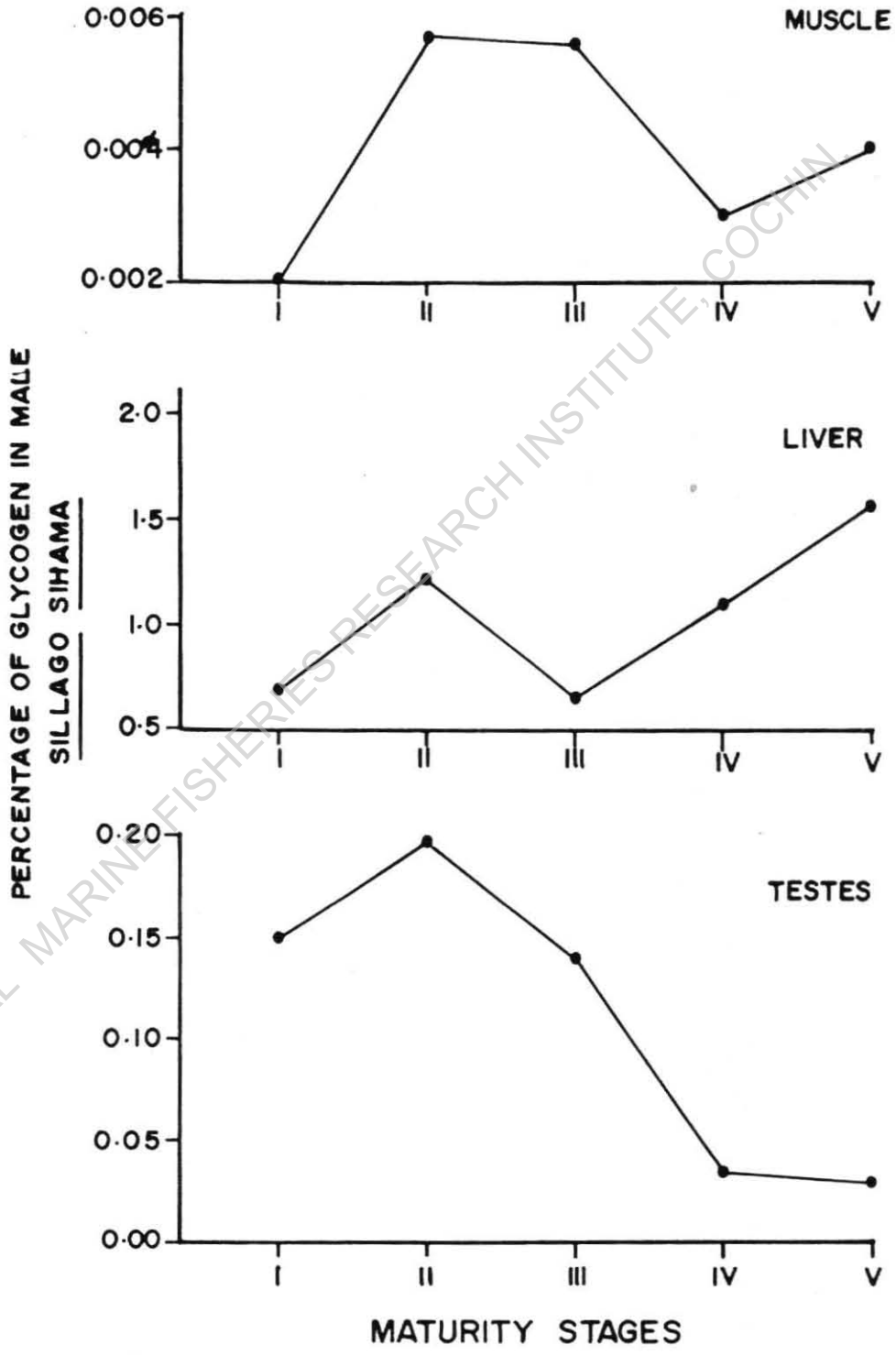


PLATE XXXII

Variation of glycogen content in the muscle, liver and testes of *S. sihama* during different maturity stages.

PLATE XXXII



On dry weight basis, the trend in variation was almost similar with a range of 0.009-0.026%.

Lipid:

Lipid content in the muscle varied from 0.43% to 1.9%. There was a sharp decrease from stage I to stage II and gradual increase till stage V (Plate XXXIII). On dry weight basis, muscle lipid content decreased from 5.96% in stage I to 1.8% in stage II and steadily increased to 8.35% in stage V.

Cholesterol:

The cholesterol content in the muscle exhibited a steady decrease from 0.16% in stage I to 0.027% in stage IV and an increase to 0.061% in stage V (Plate XXXIV). A similar trend in variation was observed on dry weight basis, with a range of 0.121-0.724%.

Liver

The data on the biochemical composition of fresh liver during different stages of maturity are given in the Table 32 and the values estimated on dry weight basis are given in the Table 35.

Moisture:

Moisture content in the liver registered steady increase from 68.64% in stage I to 77.24% in stage V (Plate XXIX).

Total Carbohydrates:

Carbohydrate content in the liver varied between 3.78 and 10.07. The values were comparatively higher in stages I and II (Plate XXX). On dry weight basis, carbohydrate content increased from 25.67% in stage I

PLATE XXXIII.

Variation of lipid content in the muscle, liver and testes of *S. sihama* during different maturity stages.

PLATE XXXIII

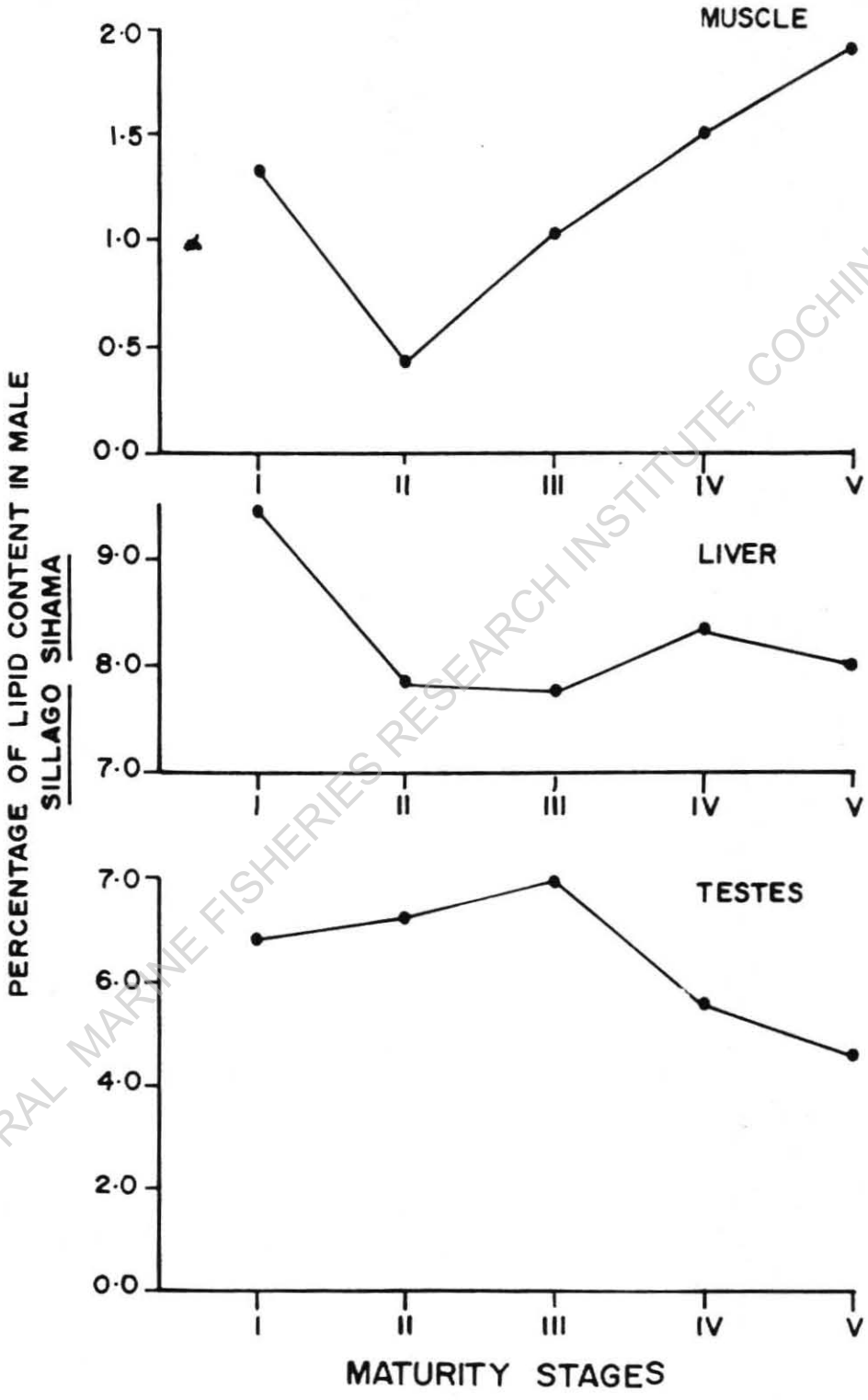


PLATE XXXIV.

Variation of cholesterol content in the muscle, liver and testes of *S. sihama* during different maturity stages.

PLATE XXXIV

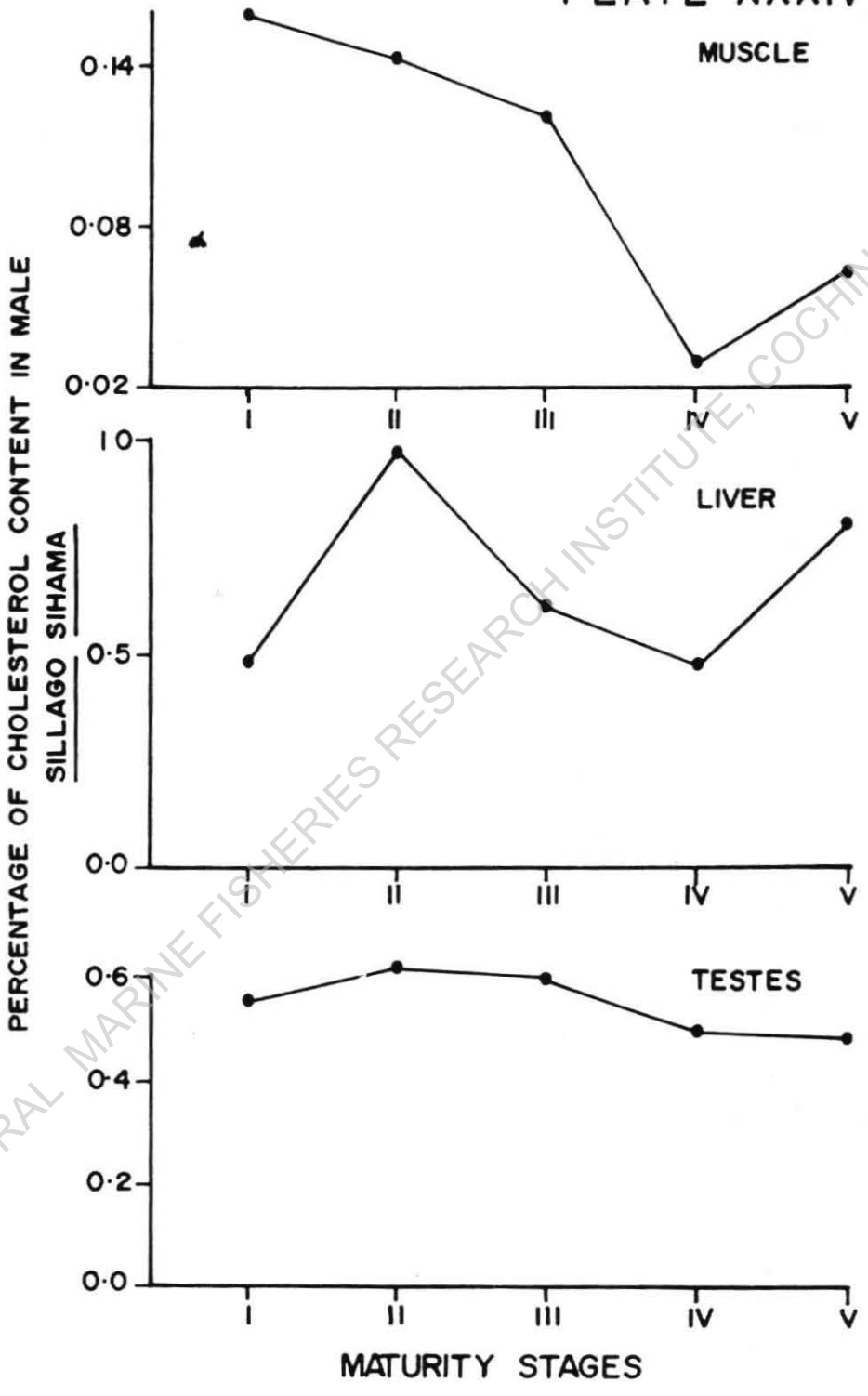


TABLE 32. Biochemical composition of fresh liver tissue of *S. sihama* during different stages of maturity

Stages	Moisture %	Total Carbohydrates %	Protein %	Glycogen %	Lipid %	Cholesterol %
I	68.64 ± 1.00	8.05 ±1.00	15.14 ±1.00	0.70 ±0.15	9.47 ±1.50	0.483 ±0.055
II	70.65 ±0.52	10.05 ±2.00	14.71 ±0.01	1.20 ± 0.15	7.84 ±2.00	0.969 ±0.027
III	70.96 ±1.50	3.92 ±0.09	12.63 ±0.03	0.65 ±0.10	7.74 ±1.20	0.612 ±0..024
IV	73.40 ±1.50	3.78 ±0.09	13.86 ±0.10	1.10 ±0.10	8.37 ±1.44	0.476 ±0.100
V	77.24 ±2.50	4.71 ±1.32	11.01 ±0.10	1.60 ±0.20	8.00 ±1.00	0.796 ±0.065

to 34.75% in stage II, then decreased to 15.67% in stage IV and further increased to 17.62% in stage V.

Protein:

The range of protein content in the liver was 11.01-15.14%. Maximum level was observed in stage I. Protein content decreased steadily from stage I to stage III and after increasing in stage IV, again decreased in stage V (Plate XXXI). On dry weight basis, the trend in variation was almost similar with a range of 44.76-60.9%.

Glycogen:

Glycogen content in the liver varied from 0.65 to 1.56%, with lowest value in stage III. Glycogen content values were relatively higher in stage IV and V (Plate XXXII). On dry weight basis, glycogen content increased from 0.22% in stage I to 0.42% in stage II, decreased to 0.22% in stage III, and further increased to 0.68% in stage V. During all stages of maturity, the glycogen content in the liver of male fish was more than that of the female fish.

Lipid:

The range of lipid content in the liver was 7.74-9.47%. As in the case of the protein, lipid content in the liver showed decreasing trend from 9.47% in stage I to 7.74% in stage III and an increase to 8.37% in stage IV (Plate XXXIII). On dry weight basis, the lipid content showed decrease from 30.55% in stage I to 28.67% in stage III and an increase to 32% in stage V.

Cholesterol:

Cholesterol content in the liver varied between 0.476 and 0.969% . It increased markedly from stage I to stage II, decreased till stage IV and further increased in stage V (Plate XXXIV). A similar trend in variation was observed on dry weight basis, with a range of 1.54-3.3%.

Testes

The data on the biochemical composition of fresh testes during different stages of maturity are given in the Table 33 and the values estimated on dry weight basis are given in the Table 35.

Moisture:

Moisture content increased steadily from 77.5% in stage I to 79% in stage IV and decreased to 77.5% in stage V (Plate XXIX).

Total carbohydrates:

The range of carbohydrate content in the testis was 1-3.37%. It increased slightly from 3% in stage I to 3.7% in stage II and thereafter decreased steadily to 1% in stage V (Plate XXX). On dry weight basis, a similar trend in variation was observed, with a range of 5.57-16.27%.

Protein:

Protein content in the testes varied from 6-7.43%. The variation during different stages of maturity was less marked (Plate XXXI). On dry weight basis, the protein content increased from 26.67% in stage I to 38.43% in stage IV and decreased to 31.03% in stage V.

TABLE 37. Biochemical Composition of Blood plasma of male *S. sihama* during different stages of maturity

Stages	Glucose (mg/100 ml)	Protein (g/100 ml)	Lipid (g/100 ml)	Cholesterol (mg/100 ml)
I	275.61 ±15.65	9.00 ±0.25	4.50 ±0.20	300.50 ±25.50
II	405.68 ±25.75	9.30 ±0.30	4.00 ±0.10	290.35 ±15.00
III	400.50 ±25.00	6.00 ±0.10	4.00 ±0.2	190.45 ±10.00
IV	433.40 ±15.65	2.80 ±0.10	3.50 ±0.10	142.05 ±15.65
V	200.50 ±25.00	4.50 ±0.20	4.50 ±0.20	276.36 ±25.00

TABLE 37. Biochemical Composition of Blood plasma of male *S. sihama* during different stages of maturity

Stages	Glucose (mg/100 ml)	Protein (g/100 ml)	Lipid (g/100 ml)	Cholesterol (mg/100 ml)
I	275.61 ±15.65	9.00 ±0.25	4.50 ±0.20	300.50 ±25.50
II	405.68 ±25.75	9.30 ±0.30	4.00 ±0.10	290.35 ±15.00
III	400.50 ±25.00	6.00 ±0.10	4.00 ±0.2	190.45 ±10.00
IV	433.40 ±15.65	2.80 ±0.10	3.50 ±0.10	142.05 ±15.65
V	200.50 ±25.00	4.50 ±0.20	4.50 ±0.20	276.36 ±25.00

TABLE 35. Biochemical composition of muscle, liver and testes of male *S. sihama* on dry weight basis during different stages of maturity

Stages	MUSCLE					LIVER					TESTES				
	Total Carbohydrates	Protein %	Glycogen %	Lipid %	Cholesterol %	Total Carbohydrates %	Protein %	Glycogen %	Lipid %	Cholesterol %	Total Carbohydrates	Protein %	Glycogen %	Lipid %	Cholesterol %
I	2.56 ±1.02	63.34 ±0.86	0.009 ±0.000	5.96 ±0.43	0.724 ±0.210	25.67 ±3.19	48.28 ±3.19	2.20 ±0.48	30.55 ±4.84	1.50 ±0.18	13.33 ±0.89	26.67 ±1.60	0.670 ±0.004	31.56 ±0.95	2.40 ±0.30
II	1.82 ±0.40	60.58 ±0.77	0.024 ±0.004	1.80 ±0.34	0.604 ±0.064	34.75 ±6.80	49.50 ±0.31	4.20 ±0.50	26.13 ±2.80	3.30 ±0.09	16.27 ±0.45	31.23 ±1.59	0.740 ±0.090	32.00 ±0.40	2.80 ±0.40
III	1.82 ±0.40	58.87 ±0.43	0.026 ±0.000	4.67 ±0.46	0.501 ±0.114	16.25 ±0.31	44.76 ±0.10	2.20 ±0.30	28.67 ±4.40	2.11 ±0.08	11.78 ±0.43	33.16 ±1.66	0.640 ±0.050	35.93 ±1.40	2.70 ±0.03
IV	1.74 ±0.40	63.47 ±4.30	0.013 ±0.004	6.59 ±2.20	0.121 ±0.040	15.67 ±0.30	60.90 ±0.44	4.80 ±0.30	33.48 ±5.76	1.97 ±0.50	8.75 ±0.60	38.43 ±1.67	0.160 ±0.040	24.57 ±0.70	2.28 ±0.20
V	3.23 ±1.00	71.82 ±6.40	0.017 ±0.004	8.35 ±2.00	0.275 ±0.040	17.62 ±5.80	48.38 ±0.40	6.80 ±0.90	32.00 ±4.00	3.29 ±0.29	5.57 ±0.46	31.03 ±1.30	0.140 ±0.040	20.11 ±1.90	2.23 ±0.23

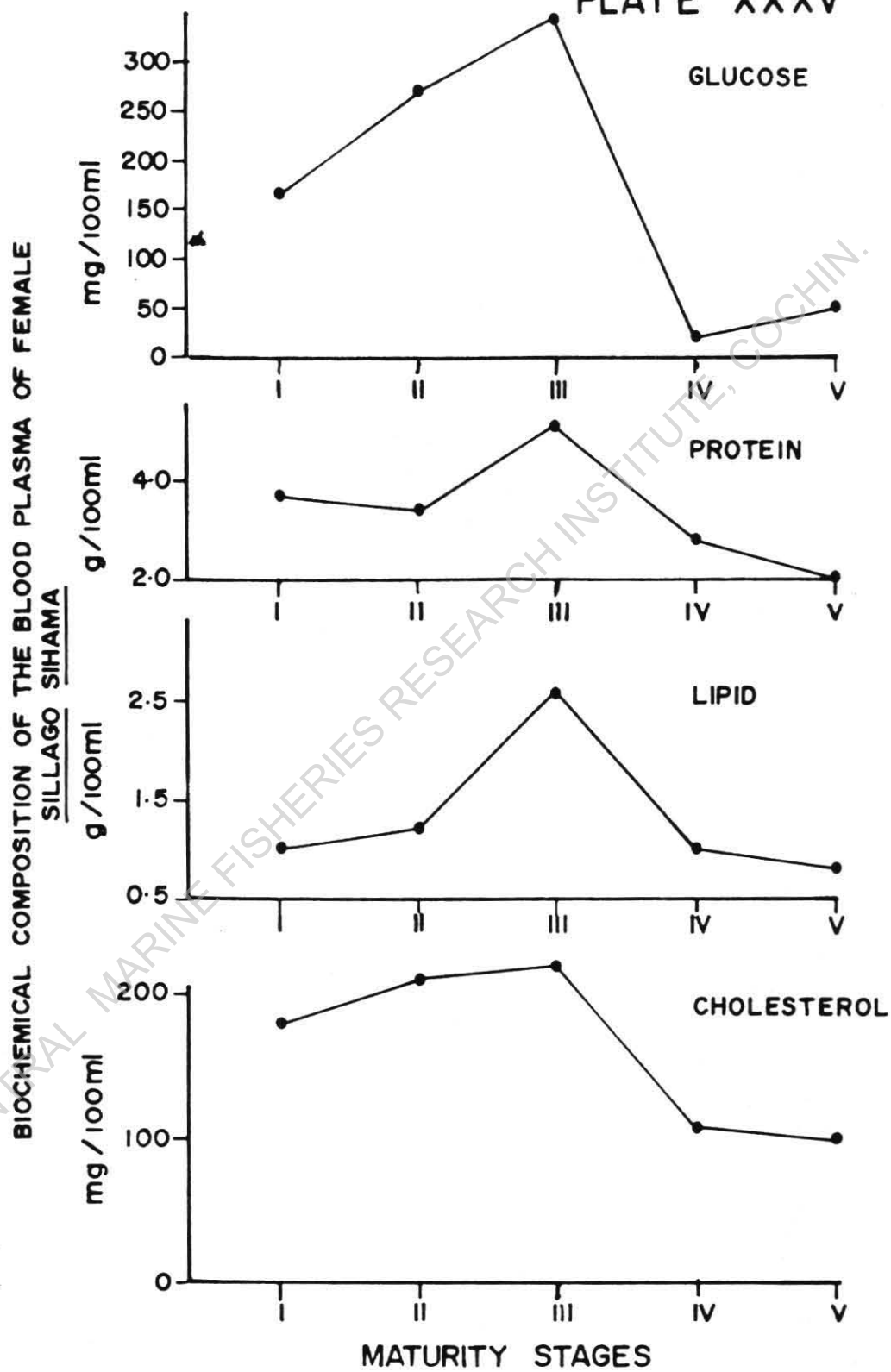
TABLE 36. Biochemical composition of blood plasma of female *S. sihama* during different stages of maturity

Stages	Glucose (mg/100ml)	Protein (g/100 ml)	Lipid (g/100 ml)	Cholesterol (mg/100 ml)
I	165.69 ±20.50	3.71 ±0.25	1.0 ±0.1	180.53 ±25.50
II	267.47 ±15.65	3.36 ±0.20	1.2 ±0.0	211.42 ±14.75
III	349.31 ±20.75	5.13 ±0.10	2.6 ±0.1	220.47 ±30.65
IV	17.60 ±4.35	2.77 ±0.35	1.0 ±0.1	106.06 ±15.65
V	50.75 ±8.35	2.00 ±0.10	0.8 ±0.0	100.60 ±15.50

PLATE XXXV.

Variations of glucose, protein, lipid and cholesterol levels in the blood plasma of female *S. sihama* during different maturity stages.

PLATE XXXV



Glycogen:

Glycogen content in the testes ranged between 0.03 and 0.164%. After a slight increase in stage II, the glycogen content decreased steadily till stage V (Plate XXXII). On dry weight basis, the variation in testes glycogen content showed the same trend.

Lipid:

Lipid content in the testes increased from 6.84% in stage I to the maximum level in this organ, namely 7.93% in stage III and thereafter declined to 4.6% in stage V (Plate XXXIII). On dry weight basis, a similar pattern of variation was observed in the lipid content, with a range of 20.11-35.93%.

Cholesterol:

Cholesterol content in the testes varied from 0.48 to 0.611%. It increased from 0.551% in stage I to 0.611% in stage II and decreased progressively to 0.48% in stage V (Plate XXXIV). On dry weight basis, the variation exhibited a similar trend with a range of 2.23-2.84%.

Blood Plasma

Data on the biochemical composition of blood plasma of male *S. sihama* during different stages of maturity are given in the Table 37 and depicted in Plate XXXVI.

Blood glucose content in male fish varied from 200.5 mg/100 ml to 433.4 mg/100 ml. Glucose content increased sharply from stage I to II and remained high till stage IV. It registered a marked decline in stage V.

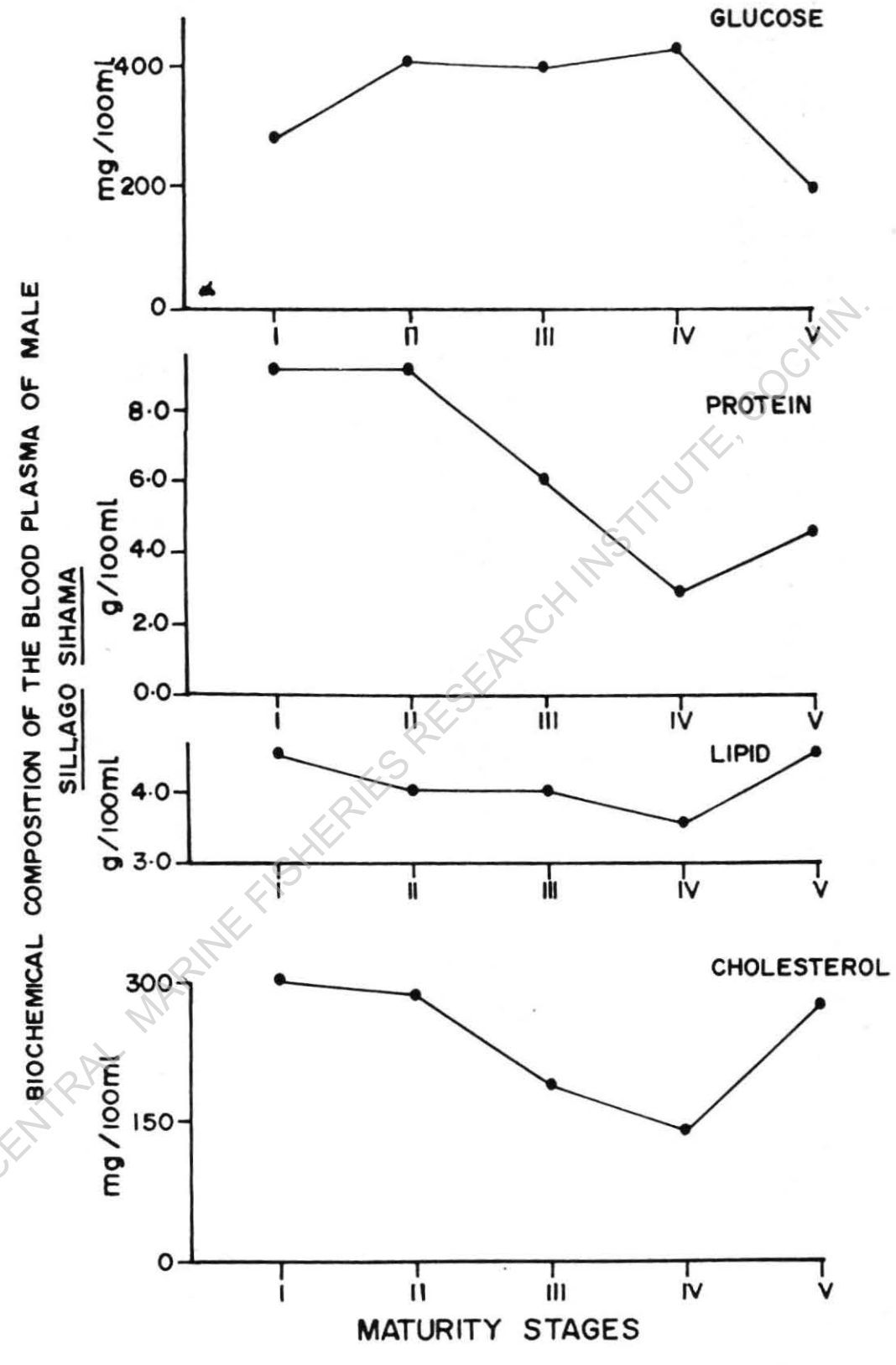
TABLE 37. Biochemical Composition of Blood plasma of male *S. sihama* during different stages of maturity

Stages	Glucose (mg/100 ml)	Protein (g/100 ml)	Lipid (g/100 ml)	Cholesterol (mg/100 ml)
I	275.61 ±15.65	9.00 ±0.25	4.50 ±0.20	300.50 ±25.50
II	405.68 ±25.75	9.30 ±0.30	4.00 ±0.10	290.35 ±15.00
III	400.50 ±25.00	6.00 ±0.10	4.00 ±0.2	190.45 ±10.00
IV	433.40 ±15.65	2.80 ±0.10	3.50 ±0.10	142.05 ±15.65
V	200.50 ±25.00	4.50 ±0.20	4.50 ±0.20	276.36 ±25.00

PLATE XXXVI.

Variations of glucose, protein, lipid and cholesterol levels in the blood plasma of male *S. sihama* during different maturity stages.

PLATE XXXVI



Protein content in blood plasma varied between 2.8-9.3 g/100 ml. It was high in stage I and stage II and decreased to the minimum level in stage IV, followed by a rise in stage V. Lipid content varied slightly during different stages of maturity; the range was 3.5-4.5 g/100 ml. It decreased from 4.5 g/100 ml in stage I to 3.5 g/100 ml in stage IV and rised to 4.5 g/100 ml in stage V. Cholesterol content exhibited a steady decline from 300.5 mg/100 ml in stage I to 142.05 mg/100 ml in stage IV and a rise to 276.36 mg/100 ml in stage V.

DISCUSSION

It is well known that during maturation and spawning activity, energy requirements of fish are on the increase, which bring about significant changes in various biochemical constituents in its body. This increasing demand for energy is caused by energy use during the formation of gonadal products.

Moisture forms the major constituent in animal body and plays decisive roles in most biochemical functions such as regulation of osmotic functions and as medium through which nutrients and other biochemical constituents are transported to various body parts. The amount of moisture in fish body is higher than that of all higher vertebrates. An animal may lose practically all of its fat and half its proteins and live, but loss of only 10% of its water causes death (Maynard and Loosti, 1962).

Moisture content of any organism varies in accordance with its various physiological activities, and usually an equilibrium is established between water and other component systems. For instance, an inverse relationship between water and lipid content in teleost fishes has been reported (Hart *et al.*,1940; Brandes and Diefrich 1958; Idler and Bitners, 1959; Jafri and Khawaja, 1970; Groves, 1970; Denton and Yosef, 1976; Pandey *et al.*, 1976; Grayton and Beamish, 1977; Shubina and Rychagona, 1981; Reinitz, 1983; Weatherley and Gill, 1983; Somvanshi, 1983; Sivakami, 1986). The sum total of these two constituents are reported to be approximately constant at any phase of maturation in fishes (Love 1970).

In the present study, the muscle moisture content of the female showed an initial increases from stage I to stage II, followed by steady

decline till stage V. Liver moisture content registered progressive decline from stage I to stage IV and an increase in stage V. Liver seems to have low moisture content, probably because it forms the storage organ for lipid, protein and carvohydrate. Ovarian moisture content showed decrease till stage III and a steap rise in stage V.

The present results indicate that in the female, the inverse-relationship of the moisture content with protein and lipid exists only in the ovary throughout the reproductive cycle, while in the muscle and liver such a relationship was not constant in all the maturity stages.

In the male, relatively higher values of muscle moisture content have been noticed in the advanced stages of maturity. Liver moisture content showed steady increase from stage I to stage V, while in the testis, the moisture content showed gradual increase from stage I to stage IV, accompanied by a decline in stage V.

In male fish, except in liver, the inverse-relationship of moisture with other components was not apparent in the other tissues. The fact that the rise in moisture content during the maturation of testis was accompanied by accumulation of the biochemical components suggests that hydration and accumulation of resources could take place as two independent events. The increase in the testis moisture content in the final stage of maturation might be for facilitating the formulation of free running milt during spawning.

The lack of a well defined inverse-relationship of the moisture content with other components in most tissues of *S.sihama* gains support from the observation of Bogveiki and Trzesinzki (1950) and Kordyl (1951) that the close

interrelationship between moisture content and the amounts of fat and protein do not show up as distinctly in non-fatty fishes such as cod, haddock, pollock etc as in the fatty fishes. *Sillago sihama* is known to be a 'lean' fish (The Wealth of India, 1962, as quoted by Pandey *et al.*, 1976).

The complex role played by protein during the different phase of growth and reproduction in fish have been discussed by several workers. Hickling (1930)., Love and Robertson (1967) and Iles (1974) have shown that the protein synthesised and accumulated in the somatic tissue during the prematuration period is utilised for gamete formation in addition to the growth of the fish. Muscle protein value was found increasing as maturation progressed in *Ophiocephalus punctatus* (Jafri and Khawaja, 1970), *Clarias batrachus* (Yagano Bano, 1977). *Esox lucius* (Medford and Mackay, 1978), *Labeo gonius* (Jain and Singh, 1981). *Rasbora danicoinus* (Sivakami, 1981) and *Cyprinus carpio* (Sivakami *et al.*, 1986). But muscle protein content appeared to decline with advancement of maturation in *Clupea harengus* (Bruce, 1924; Lovern and Wood, 1937), *Gadus morhus* (Dambas, 1964) and *Cyprinus carpio* (Masurekar and Pai, 1979).

In the present study, the protein in the muscle of female fish was found at relatively higher levels in the immature and the early maturing stages. As the maturation of ovaries advanced, the protein level decreased in the muscle at the same time showing a steady build up in the ovaries. This pattern of distribution of protein level suggests that the protein gets accumulated in the muscle tissue in the early stages of maturation and subsequently gets translocated to the ovaries as maturation process advances.

In the male, muscle protein content decreased slightly from stage I to stage III and rose in subsequent stages. However, the variations were only within a small range of 14.08 - 16.19 per cent.

Liver protein content in the female exhibited a steap rise from stage I to II, followed by gradual decline till stage IV and a further increase in stage V. Increase in protein concentration in the liver in stage II may be related to the accumulation of yolk precursors. The progressive decline of protein content in the advanced stages of maturation indicates mobilization toward gonad development. There is re-build up of protein during the partially spent condition (stage V). The increase of ovarian protein content between stage II and stage III is corroboratory of the pattern of protein translocation in the liver.

In the male, liver protein content showed gradual decrease from stage I to III, followed by increase in stage IV and further decrease in stage V. This pattern of variation is suggestive of it being utilized for germ building, as evidenced by the steady increase of testis protein content during this period.

Ovarian protein content increased steadily from stage I to III and gradually decreased in the subsequent stages. This observation is in conformity with that made in other teleosts (Ehlebracht, 1973; Medford and Mackay, 1978; Kapur, 1980; Sivakami, 1981; Nauriyal and Singh, 1985; Sivakami 1986). In the testes, protein content showed steady increase from stage I to IV and decrease in stage V.

The carbohydrate stored in the form of glycogen contributes to about 0.5-1.5 percent of the body composition of the fish. But as it is readily mobilized and is highly reducible, it forms an important source of energy (Shulman, 1974). Carbohydrate allocation during reproductive cycle has been studied in *Oncorhynchus tschawytscha* (Greene, 1926), *Salmo salar* (Belding; 1934; Fontaine and Hatey, 1953; Chang and Idler 1960) *Clarias lazera* (Yanni, 1961),*Lamptera fluviatilis* (Bentley and Follet, 1965), *Ophiocephalus*

striatus (Venugopalan, 1962), *Cirrhinus mrigala* (Lal, 1963), *Rasbora daniconius* (Sivakami, 1981), *Puntius chilinoides* (Nauriyal and Sing, 1985), *Anabas testudineus* (Dasgupta and Sircar, 1986) , *Cyprinus carpio* (Sivakami *et al.*, 1986)and *Mugil cephalus* and *Liza parsia* (Joseph, 1987).

The values of total carbohydrate estimated in the present work showed their general depletion in the muscle and liver with the advancement of maturation in both sexes. In the ovary, carbohydrate deposition till stage III is clearly evident. However, in the testes, total carbohydrate content increased only upto stage II, after which there was a steady decline till stage V.

Muscle glycogen content in female *S.sihama* exhibited slight increase from stage I to III, followed by decrease in stage IV and further increase in stage V. This observation is in conformity with that made in *Garra mullya* (Somvanshi, 1983) and *Cyprinus carpio* (Sivakami *et al.*, 1986). In the male fish too, muscle glycogen content declined in stage IV. This could be due to the utilization of glycogen reserves during the final maturation and spawning act in both sexes of *S. sihama*.

The liver glycogen content of both sexes did not show any definite pattern of variation. In the female, liver glycogen decreased from stage I to stage II, again increased in stage III and further gradually decreased in the subsequent stages. From stage I to stage II, there was an increase in ovarian glycogen content, which may indicate its mobilization from liver. However, in the subsequent stages, a similar pattern of variation was seen in the glycogen content of both liver and ovaries.

In the male too, an alternating pattern of variation was seen during the reproductive cycle. The sharp decline in glycogen level of male fish liver between stage II and stage III could not be due to the translocation of this reserve, since during this period, testes glycogen content was seen decreasing.

In spawning *Salmo salar*, average value of liver glycogen were 24.9 mg/g for male and only 0.5 mg/g for the female fish (Fontaine and Hatey, 1953). In the present study also more depletion of female fish liver glycogen content compared to that of male was evident. However, the fluctuation of glycogen level in the liver of *S. sihama* is no so indicative as to reckon it as a very decisive factor associated with spawning. Sivakami (1981) has made a similar observation in *Rasbora daniconius*.

In the present work, both ovarian and testicular glycogen levels were found to be high in the early maturation stages and declined the final maturation stages. This observation agrees with that made in other teleost fishes. Green (1926) and Chang and Idler (1960) have stated that glycogen and glucose tend to accumulate in the ovary during maturation. Sugar content in the gonads of *Cirrhinus mrigala* (Lal, 1963) and *Puntius chilinoids* (Nauriyal and Singh, 1985) was found to be maximum in the mature stage, thereafter declining during the spent stage. Glycogen content in carp oocytes increased with the advancement of maturity (Danilenko, 1971). Dasgupta and Sircar (1986) found increase in ovarian glycogen level during prespawning season in *Anabas testudineus*, Sivakami *et al.* (1986) observed similar pattern of variation in the ovarian glycogen in *Cyprinus carpio*.

In both ovaries and testes of *S. sihama* the pattern of variation of glycogen content was similar to that of the total carbohydrate content.

During gonadal maturation and spawning, the lipid material of the fish, is utilized primarily for three purposes, namely (1) as an endogenous source of energy for sustaining the fish, since several of them are known to abstain from feeding during spawning and for increased muscular activity of the fish that have spawning migratory behaviour; (2) for the synthesis of generative materials (eggs and sperms), and yolk deposition and (3) for the synthesis of steroid hormones.

The fat accumulates in various organs prior to the maturation of gonads in many fishes (Hoar, 1957). In general, lipid content in fishes increase during the maturation and registers minimum value during the peak spawning period (Milroy, 1908; Bruce, 1924; Channon and Saby, 1932, Lovern and Wood, 1937; Wilson, 1939; Rao 1967; Banerjee and Baguchi, 1970; Shchepkin, 1971; El Maghraby, 1972; Pandey *et al.*,1976).

As regarding the pattern of lipid variation in the muscle during maturation, contrasting views exist. While in several species, fat has been reported to accumulate in the muscle during maturation (Bruce, 1924; Black and Schwartz, 1950; Chidambaram *et al.*, 1952; Durairaj, 1962; Rao, 1967; Yagano Bano, 1977; Medford and Mackay, 1978; Shubina and Rychagona, 1981; Somvanshi, 1983; Sivakami *et al.*, 1986), in others depletion of muscle fat has been observed during maturation (Milroy, 1989; Dambergs, 1934; Masurekar and Pai, 1979; Sivakami, 1981). Idler and Bitners (1960) observed that in *Oncorhynchus nerka* 9 percent of muscle lipids is transferred to the ovary in female, while only 0.5 percent is transferred to the testes

in male. Thurston and Newman (1962) calculated the degree of lipid depletion in this species and found that the lipids in the white muscle drop from 9.7 to 1.8 percent while in the dark muscle from 27.4 to 6.8 percent. However, degree of decreases were of similar proportion in both the tissues.

In the present work, muscle lipid content of the female showed a steady decline from stage I to IV and a rise in stage V. This indicates its mobilization towards gonad development.

Muscle lipid content of the male showed a sharp decrease in Stage II, but otherwise exhibited higher values in other stages of maturity. It may be assumed that the translocation of muscle fat takes place only in the early maturing phase in male *S. sihama*. A similar observation was made in *Cyprinus carpio* by Sivakami *et al.* (1986).

During maturation, liver lipid content has been reported to decrease in some fishes (Shchepkin, 1979; Singh and Singh, 1984), while in other fishes the reverse trend has been noticed (Takashima *et al*., 1971; Turuk, 1972). In the present study, the liver lipid content of the female registered decrease till the third stage and a sharp rise in the subsequent stages. Mobilization of liver lipid to the gonad may be evidenced by the fact that ovarian lipid level exhibits increase between stage I and stage III.

In male, the range of variation in liver lipid content was smaller. There was a decreasing tendency from stage I to III, followed by increase in stage IV and a slight decrease in stage V. This might indicate that in male fish liver, lipid translocation takes place earlier than that in the female.

In *Cirrhinus mrigala* (Jafri, 1968; Singh and Singh, 1984) and *Clarias batrachus* (Singh and Singh, 1983), ovarian lipids have been found to increase rapidly during ripening and reach maximum value during the peak spawning period. But in *Hypophthalmus edentatus*, Carvalho (1980) observed decline in ovarian lipids during maturation. The pattern of variation in both ovarian and testicular lipid content were similar in the present study. There was gradual build-up of lipid content till stage III and subsequent decrease till Stage V. The results of the present study thus suggest that gonadal maturation in *S. sihama* is an enery consuming process and draws upon the lipid reserves, which form the chief endogenous source of energy.

Cholesterol forms the most prominent sterol in the vertebrate cells. The common precursor of all steroid hormones, particularly of androgens and oestrogen, pregnenolone, is formed from cholesterol (Dorfman and Ungar, 1965). Several workers have also described the role of cholesterol being a precursor for steroidogenesis (Krum *et al.*,1964; Major *et al.*,1967; Sandor, 1979). Cholesterol is present in the steroidogenic glands and the tropins are known to affect the tissue concentration of this sterol in the target organs (Sayers *et al.*,1944; Levin and Jailer, 1948). In several teleosts, it has been shown that estrogens stimulate the synthesis of the serum yolk precursor vitellogenin (Ho and Vanstone, 1961; Plack *et al.*, 1971; Campbell and Idler, 1976; Emmersen and Peterson, 1976; Hara and Hirai, 1978). Correlation between the annual breeding cycle and the cycle in testicular androgens have been demonstrated in a number of teleosts (Gottfried and Van Mullem, 1967; Idler *et al.*,1971; Schreck and Hopwood, 1974; Billard *et al.*, 1978).

Muscle cholesterol content has been reported to be low during the active spawning season in *Clarias batrachus* (Yagano Bano and Hameed, 1979) and in *Labeo gonius* (Jain *et al.*, 1984). In *Oncorhynchus nerka* Idler and Bitners (1955) have observed slight increase in the muscle cholestrol during maturation. These workers consider that muscle cholesterol is not used for energy purpose. A similar situation seems to exist in female *S. sihama*. Muscle cholesterol content of female fish varied hardly during the different stages of maturity.

However, in the male, cholesterol content exhibited a steady decline from stage I to stage IV, followed by a slight increase in stage V, indicating its translocation during active gonadal maturation period.

In *Cirrhinus mrigala* Singh and Singh (1984), have observed low liver cholesterol content during spawning phase and high during prespawning phase. In the present work, in female fish liver cholesterol content decreased from stage I to III and registered increase in the subsequent stages. That considerable build-up of cholesterol was seen taking place in the ovary till stage III, cleary indicates that liver cholesterol is being mobilized for gonadal maturation. The gradual increase of plasma cholesterol level during this period further supports this suggestion, for liver cholesterol is carried by blood to the ovary.

Liver cholesterol content in male *S. sihama*, after a rise in stage II, gradually decreased till stage IV and again increased in stage V. Between Stages II and IV, cholesterol translocation is likely to happen in male fish liver.

Cholesterol content of both ovary and testis exhibited rise during the early maturing stages and marked decline during the final stage of maturation and partially spent condition in several other fishes also gonad cholesterol content was observed to reach peak level during the active spawning phase (Siddiqui, 1966, 1975; Singh and Singh, 1977; Jayashree and Srinivasachar, 1979; Diwan and Krishnan, 1986). The cyclical changes in the gonad cholesterol are apparently due to the gonadal activity prior to and during the breeding season.

The low cholesterol values of gonads during the advanced maturity stages of *S. sihama* explain utilization of this component for the synthesis of steroids, which play a major role in mediating reproductive behaviour, either by action directly on brain structures governing certain behaviour patterns, or by active indirectly to influence behaviour through their effects on the development of secondary sexual characters (Liley and Stacey, 1983).

Biochemical changes in blood plasma

During sexual maturation, blood glucose level has been reported to increase (Robertson *et al.*, 1961; Nace *et al.*, 1964). The present results are in conformity with this. But in some fishes during maturation, blood glucose content was found to decrease (Jones and Macleod, 1960; Raizada and Singh, 1982). Joseph (1987) has ascribed the increase in the carbohydrate content of the blood serum in *Mugil cephalus* upto third stage of maturity to active feeding behaviour. *Sillago sihama* also is reported to feed

actively during the prespawning period (Radhakrishnan, 1957).

Love (1970) opines that serum proteins do seem show small changes with maturity, but rarely one has tried to distinguish it between maturation and asexual depletion. It is generally recognized that the protein content of fish blood changes under varying conditions, such as season, stage of maturity, spawning, type of food consumed and feeding habits (Shulman, 1974) In horse mackeral, the serum protein level was found to drop sharply during the prespawning period due to depletion for the synthesis of genital products, while in the goby, it was found increase in the prespawning period due to active feeding (Shulman, *Op. Cit*). Variations have also been found in serum proteins as related to age, diet and water temperature (Koroleva, 1963; Phillips *et al.*, 1963). Siddiqui (1977) found low values of plasma proteins in *Clarias batrachus, Heteropneustes fossilis, Ophiocephalus punctatus* and *O. striatus* during fully ripe condition, which showed diversion of protein toward gonad development. Felinska (1972) observed drop in serum proteins of *Salmo trutta* during maturation.

Plasma protein level in female *S. sihama* increased progressively and reached maximum in stage III, followed by sharp decline in the subsequent stages, Between stages II and III, plasma protein registered a marked rise and at the same time liver protein content showed decrease. This could be corroborated with the translocation of protein in the liver. Yolk protein precursors are transferred from the liver to the blood which will be subsequently

transported and deposited in the growing oocytes. The sharp decline in plasma protein content may indicate that the process of transferring proteins is slackened.

In male, the initial stages of maturity showed high plasma protein values and the advanced stages low values. Plasma protein reached the minimum value of 2.8 g/100ml in stage IV and there was a slight increase in stage V. A similar observation was made in male *M. cephalus* by Joseph (1987).

Fish blood is known to contain relatively more lipids than that of higher vertebrates (Sulya, 1960; Pickford *et al.*,1969; Takashima 1972; Perrier *et al.*,1973; Salfi *et al.*, 1976). During reproductive cycle, Serum lipids are reported to show perceptible variations (Dindo and MacGreoger 1981; Joseph, 1987).

The lipid content of the plasma showed higher values in the initial stages of maturity in female fish, while in the male, there appeared to be a slight decrease during the same period. However, in both sexes in the final maturation stage, plasma lipid values were very low.

Results of the present work are in conformity with the findings of Idler and Tsuyuki (1958), Robertson *et al.*(1961b). McCartney (1967) Felinska (1972), Lewander *et al.* (1974), Larson and Fange (1977), Dindo and McGregor (1981) and Diwan and Krishnan (1986) that during the spawning time the blood cholesterol level was reduced significantly. In both female and male *S. sihama* plasma cholesterol level showed very low value during final maturation stage. However, while in female this component showed an increasing trend in the early maturation phases, in male fish plasma cholesterol level exhibited gradual decline from stage I to IV. The increase in plasma cholesterol level in female fish during the early maturation stages is apparently due to the redistribution of cholesterol,mainly from the liver to the maturing ovary. The least values of plasma cholesterol observed in the final stages of maturity in both sexes might be due to the changes in cholesterol metabolism associated with the formation of steroid hormones, as suggested by Love (1970).

A perusal of the difference in major biochemical components between sexes of *S. shama* indicates that in general the sex differences are more prominent during the final stages of maturation. Love (1970) observed that sex differences in biochemical constituents of fishes are likely to disappear when the gonads are inactive. Jacquot (1961) has opined that differences due to sex did not exhibit a constant pattern, but are subject to seasonal variations.

In the present study, protein and lipid contents of female fish, particularly in muscle and gonad, exhibited prominently low values than that of the male fish during stages IV and V, while opposite situation

existed during the early maturation phases. This apparently suggests that female fish expends more energy during the final maturation stage. By electrophoresis, it has been shown that serum protein fractions change more profoundly in females than males (Drilhon, 1954; Thurston, 1967; Ipatov, 1970; Reshetnikov *et al.* 1980; Aida *et al.*,1973). Medford and Mackay (1978) found more liver protein content in female during prespawning phase in *Esox lucius*. Female *Trachurus trachurus* at the begining of the sexual cycle was richer in proteins than the males, while the reverse was observed after spawning (Arevalo, 1948). Belding (1934) in *Salmo salar* and Ziecik and Slawinski (1965) in *Tinca tinca* found that the female fish loses more lipid and protein than male during sexual maturation. After spawning, female *Scomber scomber* was found left with less residual muscle female was found left with less residual muscle lipid than the male (Ackman and Eaton, 1971). Results of the present work, are in conformity with these observations.

Glycogen content of all the tissues in female fish was found to be lower than that of male fish in the present study during stageIV and V, suggesting more expenditure of glycogen in the former during final maturation and spawning. This observation is in agreement with that made in Salmo salar (Fontaine and Hatey, 1953). *Gaduas morhua*(Shatunovski and Denisova, 1968) and *Heteropneustes fossilis* (Chaturvedi *et al.*,1976).

During the advanced stages of maturity, muscle cholesterol content in female *S. sihama* was found more than that in male in the present work. Jain *et al.* (1984) observed higher muscle cholesterol content in female *Labeo gonius* than that in males on the whole year basis. Ovarian cholesterol

content has sufferred comparatively more depletion during final maturity stage in *S. sihama*. This is in conformity with the observation mae in *Heteropneustes fossilis* (Chaturvedi *et al.*, 1976).

In conclusion, the present study clearly brings out the variation in the levels of major biochemical components in the different tissues of *S. sihama*. These variations seemed to be more well defined in female. The sex differences in biochemical composition were more evident during the final stages of maturation. In general, there was build-up of biochemical constituents in the muscle, liver and blood plasma during the early maturation stages followed by their translocation to the gonads for the formation of gametes and to meet energy requirements for the spawning activity.

CHAPTER VII

HISTOCHEMISTRY OF OOCYTES DURING MATURATION

Histochemical methods provide qualitative information regarding the physiological activities or state of the cells and tissues. In fish histochemical changes of gonadal tissues have been studied with a view to understanding the biochemical reaction and characterisation of tissues, cell and organelles. Histochemical changes during gonadal maturation in teleosts has been studied by several workers, such as Marza *et al.* (1937) in *Fundulus heteroclitus*, Yamamoto,T. (1955) in *Oryzias latipes*, Blanc-Livni (1971) in *Cyprinus carpio*, Beams and Kessel (1973) in *Salmo* gairdneri and Khoo (1979) in Carassius *auratus*. Recently, Mayer *et al.* (1988) have described the cytochemical changes taking place in the oocytes of sea bass, *Dicentrarchus labrax*.

Carbohydrate histochemistry of *Oryzias latipes* (Aketa, 1954; Yamamoto,T., 1958) and *Liopsetta obscura* (Yamamoto, 1956e,f) has been studied. Role of protein in yolk synthesis has been investigated in *Blennius pholis* (Shackley and King, 1977) and sheep head minnow (Selman and Wallace, 1982). Lipid composition of oocytes has been discussed by Yamamoto (1958a), Nath (1960) and Guraya (1963). Hisaoka and Firlit (1962) studied nucleic acid variations in *Brachydanio rerio*. Alkaline phosphatase and acid phosphatase activities have been reported, respectively in *Lepomis macrochirus* (Kugler *et al.*, 1956) and in *Carassius auratus* and *Cyprinus carpio* (Varo *et al.*, 1979).

Among Indian teleosts, practically all work has been done in freshwater species. Changes in ovarian histochemical characteristics of *Ophiocephalus punctatus*, *Barbus ticto* and *Boleophthalmus dussumieri* (Chopra, 1958a,b,1960),

Cirrhina mrigala (Lal, 1963), *Channa maruleus* (Guraya, 1965), *Gambusia affinis* (Verma, 1977) and *Channa punctatus* (Verma *et al.*, 1981) have been studied. Role of protein in yolk formation has been discussed in *Heteropneustes fossilis* by Nath and Sundaraj (1979). Nucleic acid and protein histochemistry of *Mastacembelus armatus* have been investigated (Saxena *et al.*, 1979). Dutt and Govindan (1967, 1969) studied lipids, polysaccharides, -SH and -SS groups of *Anabas scandens* ovary. Alkaline phosphatase activity in the ovary of this species has been demonstrated by Dutt *et al.* (1975).

A perusal of the literature of fish vitellogenesis studies by histochemical methods show numerous variations in the origin, number and chemical transformation of yolk inclusions during oocyte growth. The above mentioned works notwithstanding, the chemical composition of the various yolk inclusions is still poorly defined. Many points of difference require further elucidation. Except the work of histochemical analysis of the male reproductive system in *Mugil cephalus* and *Liza parsia* (Joseph, 1987), no other detailed histochemical studies have been carried out on Indian marine teleosts. Hence the present study on the histochemistry of carbohydrates, protein, lipid and nucleic acids in the oocytes of *Sillago sihama* has been carried out, with a view to perceiving the patterns of histochemical changes taking place in the different oocyte inclusions during oocyte growth and vitellogenesis.

HISTOCHEMICAL TESTS AND THEIR INTERPRETATION

Carbohydrates, proteins, lipid and nucleic acids are demonstrated by chromogenic agents which selectively take up the stain so as to enable their idendity. The specific tests for each type of carbohydrate, protein, lipid and

nucleic acid are given in the Table 38 and the procedures adopted for blocking the reactive groups are given in the Table 39. The intensity of staining in each case is marked by arbitrary symbols, - (no reaction), + (weak), ++ (moderate) and +++ (strong). The absence of an organelle in a oocyte stage is indicated by (*). The tests are performed from more general ones to the specific types.

Carbohydrates: Though periodic acid schiff (PAS) is commonly used as a general test for carbohydrates, all biomolecules with 1,2 glycol groups are found to respond to this test and hence a number of control slides are employed with different blocking procedures to identify the specific types of 1,2 glycols present.

Proteins: Mercuric Bromophenol blue test (MBB) is used as a general test for proteins and ninhydrin-schiff for amino groups. Specific tests for proteins are done to detect -SH groups, -SS groups, tyrosine and tryptophan. The specificity of each test for a particular reactive group is confirmed by staining control slides that are subjected to blocking procedures, that blocked the specific reactive groups.

Lipids: Histochemistry of lipids depends on the preferential solubility of the dye into the various parts of the tissue. Lipid is extracted from the control sections before staining them. A comparison between stained sections of both extracted and unextracted material indicates the type of lipids present. Sudan Black B is used as the general stain to detect lipids. Neutral lipids are detected by Oil-red-O method, while phosphlipids are specifically detected by Nile blue method.

Nucleic acids: In the Toluidine blue-methyl green-orange-G method, DNA and RNA are clearly differentiated, with the former staining light to bright green and the latter deep blue. Feulgen test is specific for DNA and it stains red-purple.

TABLE 38. Blocking reactions and extraction procedures of specific reactive groups

S.No.	Methods	Reactive Groups
1.	Acetylation	Blocking of 1,2 glycols
2.	Deactylation	Reversal of acetylation
3.	Taka diastase treatment	Removal of glycogen
4.	Deamination	Removal of amino groups
5.	Mercaptide	Blocking of -SH groups
6.	Iodination	Blocking of -OH groups
7.	40% Formaldehyde	Blocking of tryptophan
8.	Pyridine extraction	Removal of phospholipids
9.	Chloroform-methanol extraction	Removal of lipids.

TABLE 39. Histochemical tests and the corresponding reactive groups.

	Tests	Reactive Groups
CARBOHYDRATE	Periodic Acid Schiff's (PAS)	1,2 glycol groups (magenta or purple)
	Best's carmine	Glycogen (red)
	Alcian blue - PAS	Acid mucopolysaccharides (blue)
PROTEINS	Mercuric bromophenol blue(MBB)	Proteins (deep blue)
	Ninhydrin-schiff	Amino groups (Pink)
	Ferric-Ferricyanide	-SH groups (blue)
	Performic acid-Alcian blue	-SS groups (greenish blue)
	Millon's test	Tyrosil groups (red or pink)
	P-Dimethylamino-benzal-dehyde (DMAB)	Tryptophan (deep blue)
LIPIDS	Sudan Black B	Lipids (bluish black)
	Nile blue with sulphuric acid	Phospholipids (blue)
	Oil-red 'O'	Neutral lipids (red)
NUCLEIC ACIDS	Toluidine blue-methylgreen-Orange G(TMOG)	RNA (deep blue) and DNA (bright or light green)
	Feulgen test	DNA (red purple)

OBSERVATIONS

The results of various histochemical tests are indicated in Table 40 and a consolidated summary of the results is given in Table 41.

Carbohydrate histochemistry

Primary oocyte stage: 1,2 glycol groups, glycogen and acid mucopolysaccharides were absent in the ooplasm, nucleoplasm, nuclear membrane and nucleolus of the primary oocytes.

Vacuolated oocyte stage: Follicular epithelium did not contain carbohydrates. Zona radiata externa, which is the first formed ZR layer, showed moderate response to the staining for 1,2 glycol groups and weak response to that for glycogen. The perinuclear ring of vacuoles (oil droplets) did not contain polysaccharides. Similarly, nucleus and its components also showed negative response to carbohydrates. Acid mucopolysaccharides were absent in all oocyte constituents.

Yolk granule oocyte stage: (Plate XXXVII Figs. 1 & 2): Follicular epithelium did not stain for 1,2 glycol groups and Best's carmine test for glycogen. Zona radiata externa contained high concentration of 1,2 glycol groups and traces of glycogen, whereas zona radiata interna contained only traces of 1,2 glycol groups and glycogen. Yolk vesicles (cortical alveoli) showed strong positive response to PAS-staining, while weak reaction for glycogen staining. Mucopolysaccharides were absent in all the constituents of oocyte. Oil droplets, yolk granules, and germinal vesicle did not contain carbohydrates.

TABLE 40. Histochemical characterisation of different oocyte stages of *Sillago sihama*

	Histochemical Tests	Primary Oocyte Stage				Vacuolated Oocyte Stage						Yolk Granule Oocyte Stage								
		OP	NP	NM	NO	FE	ZRE	PNV (=OD)	NP	NM	NO	FE	ZRE	ZRI	YV	YG	OD	NP	NM	NO
CARBOHYDRATE	PAS	-	-	-	-	-	++	-	-	-	-	-	+++	+	+++	-	-	-	-	-
	Acetylation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Deacetylation	-	-	-	-	-	++	-	-	-	-	-	+++	+	+++	-	-	-	-	-
	Choloroform: methanol	-	-	-	-	-		-	-	-	-	-				-	-	-	-	-
	Taka Diastase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Best's carmine	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-
	Taka Diastase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Alcian Blue- PAS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PROTEIN	MBB	+++	+	+	++	+	+++	-	+	+	+++	+	+++	+++	+	+++	-	+	+	+++
	Ninhydrin Schiff	+++	+	+	++	+	+++	-	+	+	+++	+	+++	+++	-	+++	-	+	+	+++
	Deamination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ferric-Ferricyanide	-	-	-	-	+	++	-	-	-	++	+	++	++	+++	+	-	+	+	++
	Mercaptide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Performic acid alcian blue	-	-	-	++	-	+	-	-	-	+	-	+	+	-	+++	-	-	-	+
	Milon's test	-	-	-	+	+	++	-	-	-	+	+	++	++	-	+++	-	+	+	+
	Iodination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DMAB-Nitrite method	-	-	-	+	+	++	-	-	-	+	+	++	++	-	+++	-	-	-	-
	Fprmaldehyde	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(Cont'd.....)

TABLE 40 (Contd....)

	Histochemical tests	Primary oocyte Stage				Vacuolated oocyte stage						Yolk granule oocyte state								
		OP	NP	NM	NO	FE	ZRE	PNV (=OD)	NP	NM	NO	FE	ZRE	ZRI	YV	YG	OD	NP	NM	NO
LIPID	Sudan Black-B	++	-	-	++	-	+++	+++	+	++	++	-	+++	-	-	++	+++	-	-	-
	Pyrodine extraction	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
	Chloroform Methanol Extraction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Nile Blue Method	++	-	-	++	++	++	++	+	+	++	++	++	-	-	++	++	-	-	-
	Pyridine extraction	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
	Chloroform: Methanol Extraction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oil-Redo	++	-	-	++	++	+++	+++	+	+	++	++	+++	-	-	++	+++	-	-	-
	Pyridine extraction	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
	Chloroform: Methanol Extraction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NUCLEIC ACIDS	TMOG	+	++	+	+++	+++	+++	-	+	-	+++	+++	+++	++	-	++	-	+	-	+++
	Perchloric acid-Sodium Carbonate Solution	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Feulgen Test	-	++	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

OP = Coplasm NP = Nucleoplasm NM = Nuclear Memorane NO = Nucleolus FE = Follicular Epithelium ZRE= Zona radiata externa
PNV = Peri-nuclear vacuoles OG = Oil droplets ZRI = Zona radiata interna YV = Yolk vesicles YG = Yolk granules

TABLE 41. Summary of the histochemical analyses of different oocyte stages of *Sillago sihama*

Oocyte Constituent	Primary oocyte stage	Vacuolated oocyte stage	Yolk granule oocyte stage
Oo plasm	Carbohydrates not detected.	•	•
Follicular Epithelium	•	Carbohydrate not detected.	Carbohydrates not detected.
Zona Radiata Externa	•	Moderately positive to 1,2 glycol groups. Weakly positive to glycogen Acid Mucopoly Saccharides not detected	Strongly positive to 1,2 glycol groups. Weakly positive to glycogen acid mucopolysaccharides not detected.
Zona Radiata Interna	•	•	Weakly positive to 1,2 glycol groups and glycogen. Acid Mucopoly saccharidesnot detected.
Yolk vesicles	•	•	Strongly positive to 1,2 Glycol groups and weakly positive to glycogen acid mucopoly saccharides not detected.
Yolk granules	•	•	Carbohydrates not detected.
Oil Droplets	•	Carbohydrates not detected.	Carbohydrates not detected.
Nucleoplasm	Carbohydrates not detected.	Carbohydrates not detected.	Carbohydrates not detected.
Nuclear membrane	Carbohydrates not detected.	Carbohydrates not detected.	Carbohydrates not detected.
Nucleolus	Carbohydrates not detected.	Carbohydrates not detected.	Charbohydrates not detected.
Ooplasm	Strongly positive to general proteins and amino groups,-SH groups, S-S Groups, Tyrosine and tryptophan not detected.	•	•

(Contd.....)

TABLE 41 (Contd.....)

Oocyte constituent	Primary oocyte stage	Vacuolated oocyte stage	Yolk granule oocyte stage
Follicular epithelium	*	Weakly positive to general proteins, Amino groups, -SH groups, Tyrosine and Tryptophan. S-S groups not detected.	Weakly positive to general protein Amino groups, -SH groups, tyrosine and trypohan. S-S groups not detected.
Zona Radiata Externa	*	Strongly positive to General proteins and amino groups. Moderately positive to -SH groups, tyrosine and tryptophan. Weakly positive to S-S groups.	Strongly positive to general proteins and amino groups. Moderately positive to -SH groups, tyrosine and trypto-phan. Weakly positive to S-S groups.
Zona Radiata Interna	*	*	Strongly positive to general proteins and amino groups. Moderately positive to -SH groups, tyrosine and tryptophan. Weakly positive to S-S groups.
Yolk Vesicles	*	*	Weakly positive to General proteins. Strongly positive to -SH groups. Amino groups, S-S groups, tyrosine and tryptophan not detected.
Yolk granules	*	*	Strongly positive to general proteins, amino groups, S-S groups, tyrosine and tryptophan. Weakly positive to -SH groups.
Oil troplets	*	Proteins not detected.	Proteins not detected.
Nucleoplasm	Weakly positive to general proteins and amino groups. -SH groups, S-S groups, tyrosin and tryptophan not detected.	Weakly positive to general proteins and amino groups.-SH groups, S-S groups, tyrosine and tryptophan not detected.	Weakly positive to general proteins, amino groups, -SH groups, tyrosine and tryptophan. S-S groups not detected.

(Contd.....3)

TABLE 41. (Contd.....)

Oocyte constituent	Primary oocyte stage	Vacuolated oocyte stage	Yolk granule oocyte stage
Nuclear membrane	Weakly positive to general proteins and amino groups. -SH groups, S-S tyrosine and tryptophan not detected.	Weakly positive to general proteins and amino groups. -SH groups, S-S groups, tyrosine and tryptophan not detected.	Weakly positive to general proteins, amino groups, -SH groups, tyrosine and tryptophan. S-S groups not detected.
Nucleolus	Moderately positive to general proteins, amino groups and S-S groups. Weakly positive to tyrosine and tryptophan. -SH groups not detected.	Strongly positive to general proteins and amino groups. Moderately positive to -SH groups. Weakly positive to S-S groups, tyrosine and tryptophan	Strongly positive to general proteins and amino groups. Moderately positive to -SH groups weakly positive to S-S groups, tyrosine and tryptophan.
Ooplasm	Moderately positive to general lipids, phospholipid and neutral lipids.	*	*
Follicular epithelium	*	Moderately positive to phopholipid and neutral lipids. General lipids not detected.	Moderately positive to phospho lipid and neutral lipids. General lipids not detected
Zona Radiata Externa	*	Strongly positive to general and neutral lipids. Moderately positive to phospho lipid.	Strongly positive to general and neutral lipids. Moderately positive to phospholipid.
Zona Radiata Interna	*	*	Lipids not detected.
Yolk vesicles	*	*	Lipids not detected.
Yolk granules	*	*	Moderately positive to general lipids, phospho lipid and neutral lipids.

(Contd.....)

TABLE 41 (Contd.....)

Oocyte constituent	Primary oocyte stage	Vacuolated oocyte stage	Yolk granule oocyte stage
Oil Droplets	*	Strongly positive to general and neutral lipids. Moderately positive to phospholipid	Strongly positive to general and neutral lipids. Moderately positive to phospholipid.
Nucleoplasm	Lipids not detected.	Weakly positive to general lipids, phospholipid and neutral lipids.	Lipids not detected.
Nuclear membrane	Lipids not detected.	Moderately positive to general lipids. Weakly positive to phospholipid and neutral lipids.	Lipids not detected.
Nucleolus	Moderately positive to general lipids, phospholipid and neutral lipids.	Moderately positive to general lipids, phopholipid and neutral lipids.	Lipids not detected.
Oo plasm	Weakly positive to RNA. DNA not detected.	*	*
Follicular epithelium	*	Strongly positive to RNA. DNA not detected	Strongly positive to RNA . DNA not detected.
Zona Radiata Externa	*	Strongly positive to RNA . DNA not detected.	Strongly positive to RNA DNA not detected.
Zona Radiata Interna	*	*	Moderately positive to RNA. DNA not detected
Yolk vesicles	*	*	RNA and DNA not detected.

(Contd.....)

TABLE 41. (Contd. ....)

Oocyte constituent	Primary oocyte stage	Vacuolated oocyte stage	Yolk Granule oocyte stage
Yolk granules	*	*	Moderately positive to NA. DNA not detected.
Oil Droplets	*	RNA and DNA not detected.	RNA and DNA not detected.
Nucleoplasm	Moderately positive to RNA and DNA.	Weakly positive to RNA and DNA.	Weakly positive to RNA. DNA not detected.
Nuclear membrane	Weakly positive to RNA. DNA not detected.	RNA and DNA not detected.	RNA and DNA not detected.
Nucleolus	Strongly positive to RNA. DNA not detected.	Strongly positive to RNA. DNA not detected.	Strongly positive to RNA. DNA not detected.

PLATE XXVII.

Fig.1. Primary yolk granule oocyte; Periodic Acid Schiff.

Fig.2. Secondary yolk granule oocyte; Periodic Acid Schiff.

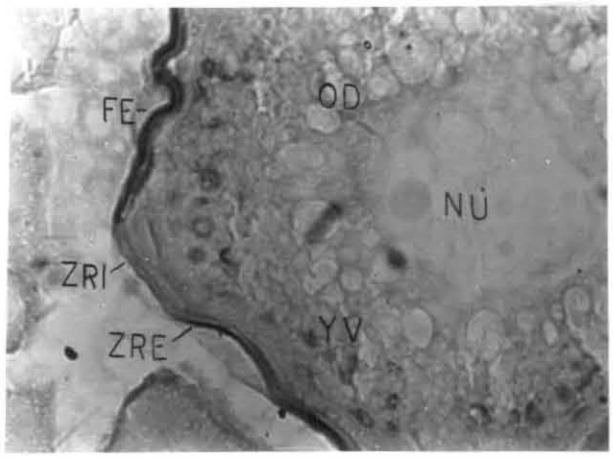
Fig.3. Transverse section of immature ovary; Sudan Black B.

Fig.4. Transverse section of mature ovary; Oil-red-O.

Fig.5. Magnified perinuclear region of Vacuolated oocyte; Sudan Black B

NU-Nucleus; OD-Oil droplets; FE-Follicular epithelium; ZRE-Zona radiata externa; ZRI-Zona radiata interna; YV-Yolk vesicles; NO-Nucleolus; IL-Intranuclear lipid bodies; VO-Vacuolated oocyte; PO-Primary oocyte; SYGO-Secondary yolk granule oocyte; TYGO-Tertiary yolk granule oocyte.

PLATE XXXVII



1

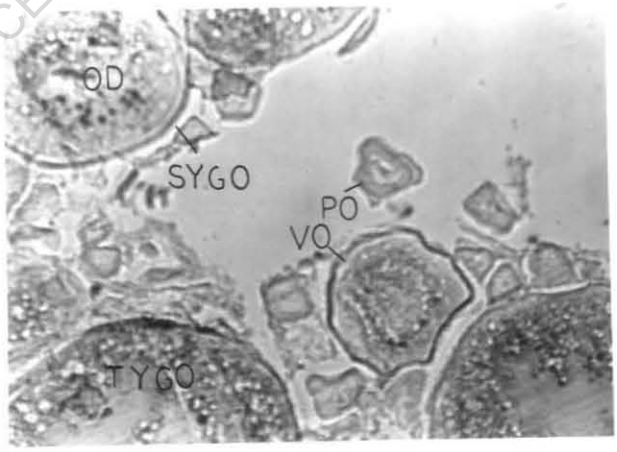
40/um

2

100/um

3

60/um



4

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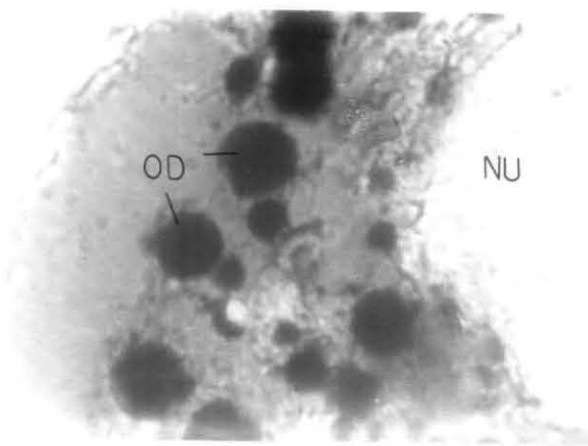


5

10/um

Control sections digested with taka diastase before staining gave a negative reaction to PAS; abolition of staining by taka diastase suggests that the yolk vesicles and zona radiata externa contain high concentration of carbohydrate, chiefly 1,2 glycol groups. Similarly, control sections which were acetylated before the test gave negative reaction to PAS and deacetylation regained the pink colour. This confirmed the polysaccharide nature of these oocyte constituents. Their positive response to carbohydrate staining after chloroform-methanol extraction further confirmed the results.

Protein histochemistry

Primary oocyte stage: General proteins and amino groups were present in high concentrations in the ooplasm, while -SH groups, S-S groups, tyrosine and tryptophan were absent. Nucleoplasm and nuclear membrane contained traces of general proteins and amino groups, while nucleolus contained moderate quantities of the same. S-S groups were present in moderate concentration in the nucleolus. -SH groups were absent in the primary oocyte nucleus.

Vacuolated oocyte stage: Follicular epithelium contained traces of general proteins, amino groups, -SH groups, tyrosine and tryptophan, whereas S-S groups were absent. Zona radiata externa contained high concentrations of general proteins and amino groups, moderate concentrations of -SH groups, tyrosine and tryptophan and traces of S-S groups. Proteins were absent in the peri-nuclear ring of oil droplets. In the nucleoplasm and nuclear membrane, general proteins and amino groups were detected in traces, while -SH groups, S-S groups, tyrosine and tryptophan were absent. High concentrations of general proteins and amino groups were detected in the nucleoli, which also contained moderate amounts of -SH groups and traces of S-S groups, tyrosine and tryptophan.

Yolk granule oocyte stage: As in the vacuolated oocytes, general proteins, amino groups, -SH groups, tyrosine and tryptophan were present in traces in the follicular epithelium, which did not show the presence of S-S groups. Zona radiata (both ZRE and ZRI) contained high concentrations of general proteins and amino groups, moderate quantities of -SH groups, tyrosine and tryptophan and traces S-S groups. Yolk vesicles showed positive response to the staining for -SH groups and a weak response for the general proteins. In contrary, yolk granule contained only traces of -SH groups, where as general proteins, amino groups, S-S groups, tyrosine and tryptophan were detected in high concentrations. Oil droplets did not contain any proteins. Both nucleoplasm and nuclear membrane showed weak reaction to the tests for general proteins, amino groups, -SH groups, tyrosine and tryptophan, while S-S groups were absent. Nucleolus stained strongly for general proteins and amino groups, moderately for -SH groups and weakly for S-S groups, tyrosine and tryptophan.

Lipid histochemistry

Primary oocyte stage: Ooplasm and nucleolus contained moderate quantities of general lipids, phospholipids and neutral lipids (Plate XXXVII, Figs. 3 & 4). Nucleoplasm and nuclear membrane did not stain for lipids. The nucleus also contained few 'intra nuclear lipid bodies', which were moderately sudanophilic and distinct from nucleoli.

Vacuolated oocyte stage: Follicular epithelium responded moderately to the staining for phospholipid and neutral lipids, while general lipids were not detected. Zona radiata externa and oil droplets (Plate XXXVII, Fig. 5) were strongly positive to general lipids and neutral lipids and moderately positive to phospholipids. Nucleoplasm contained only traces of all lipids, where as nuclear membrane was found to contain relatively more general

lipids. Nucleolus was moderately positive to general lipids, phospholipids and neutral lipids. Intra nuclear lipid bodies were detected.

Yolk granule oocyte stage: (Plate XXXVIII Figs. 1 - 3): Staining of yolk granule oocytes for lipids in the follicular epithelium, oil droplets and zona radiata externa were similar to that of vacuolated oocytes, with relatively more lipids detected in the latter two of the oocyte constituents. Unlike ZRE, the ZRI did not contain lipids. Similarly, yolk vesicles and germinal vesicle also responded negatively to lipid staining. Intra nuclear lipid bodies were not detected.

Nucleic acid histochemistry

Primary oocyte stage: Ooplasm and nuclear membrane responded weakly to RNA staining, where as DNA was not detected. Nucleoplasm contained moderate quantities of both RNA and DNA. Nucleolus stained deep blue in TMOG test, indicating high concentration of RNA; nucleolus did not stain for DNA.

Vacuolated oocyte stage: Follicular epithelium, zona radiata externa and nucleolus contained high concentrations of RNA. Nuclear membrane and oil droplets did not contain nucleic acids. Nucleoplasm responded weakly to the staining for RNA and DNA. The peri-nuclear region of the vacuolated oocytes stained strongly for RNA (Plate XXXIX, Fig. 1).

Yolk granule oocyte stage: As in the previous stage., follicular epithelium, zona radiata externa and nucleolus were highly positive to the staining for RNA. ZRI appeared to contain relatively less RNA than ZRE. Yolk vesicles, oil droplets and nuclear membrane did not show positive response to nucleic acid staining. Yolk granules contained moderate quantity of RNA. Some highly

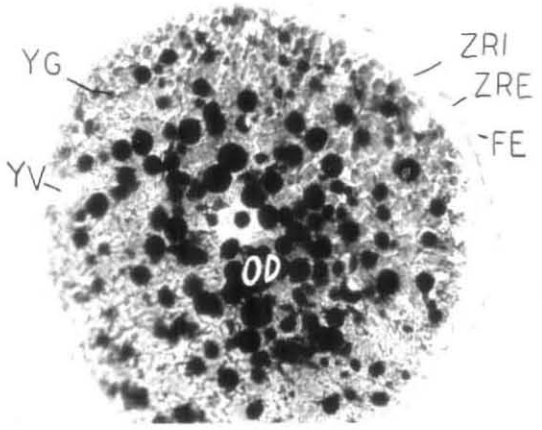
PLATE XXXVIII.

Fig.1. Tertiary yolk granule oocyte; Sudan Black B.

Fig.2. Tertiary yolk granule oocyte showing 'migratory' germinal vesicle; Sudan Black B.

Fig.3. Tertiary yolk granule oocyte; Oil-red-0

FE-Follicular epithelium; ZRE-Zona radiata externa; ZRI-Zona radiata interna; YV-Yolk vesicles; YG-Yolk granules; OD-Oil droplets; GV-Germinal vesicle.



1

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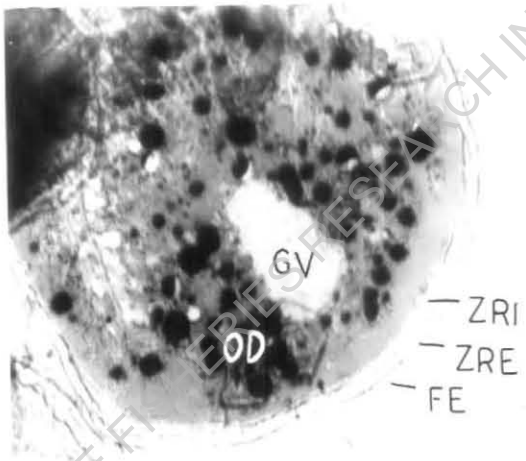
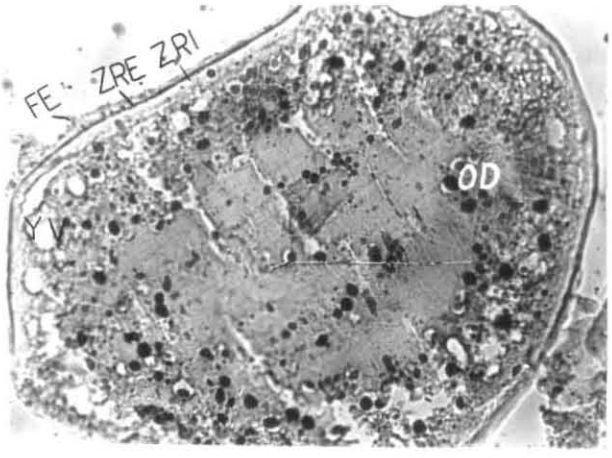


2

50 µm



3

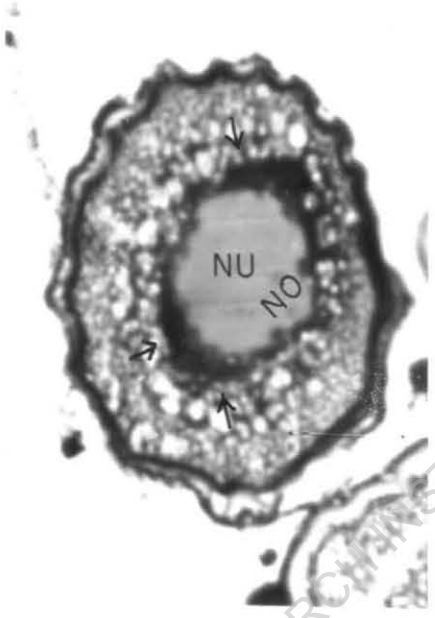
50 µm

PLATE XXXIX.

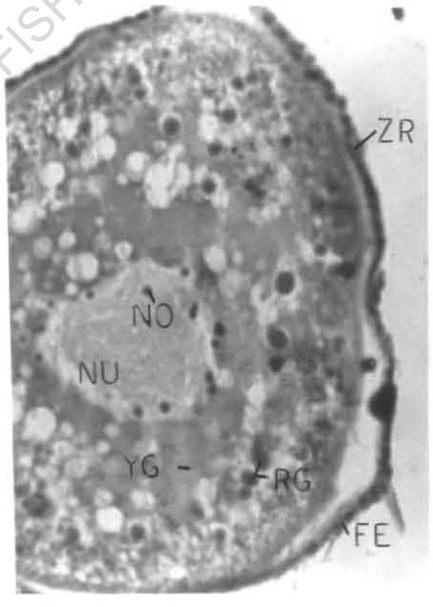
Fig.1. Vacuolated oocyte; Toluidine blue-methyl green-orange G.

Fig.2. Secondary yolk granule oocyte; Toluidine blue-methyl green-orange G.

NU-Nucleus; NO-Nucleolus; FE-Follicular epithelium; ZR-Zona radiata; YG-Yolk granules; RG-RNA Granules.



1 80 µm



2 80 µm

RNA-positive granules, distinct from yolk granules, have been found scattered in the cortical ooplasm of the yolk granule oocytes (Plate XXXIX, Fig. 2). Unlike in the previous stage, DNA was not detected in the germinal vesicle of the yolk granule oocytes.

DISCUSSION

In the chapter on biochemical analysis, it was found that ovarian maturation involves subtle changes in the body constiution with the advancement of maturation. The present histochemical studies of the oocytes during their maturation help in providing qualitative information of the biochemical changes taking place in their constituents.

Follicular epithelium

Histochemical studies have demonstrated lipid droplets (composed of phopholipids, mitochondria, golgi complex and and abundant RNA-containing basophilic substance, or ergoplasm) in the follicle cells (Guraya, 1965, 1976, 1978, 1979a; Hurley and Fisher, 1966; Ramadan *et al.*, 1979b; Guraya and Kaur, 1982; Sun and Xizai, 1983). In the present study, the follicular epithelium was found to contain little amounts of general proteins, amino groups, S-S groups, tyrosine and tryptophan, moderate quantities of phospholipids and neutral lipids and high concentration of RNA.

Various electron microscope and histochemical studies have suggested that the follicular epithelium is involved in the synthesis of proteins and lipids during the growth of oocyte (Guraya, 1986). In the present work, the strong positive response of follicular epithelium to RNA staining indicates active protein synthesis. The proteins synthesised may partly be used for the growth and maturation processes of the follicular epithelium itself, and party for their transport into the oocyte as well as the formation of zona material (Guaraya, 1986).

Zona radiata

The zona material in elasmobranchs and teleosts consists of mucopolysaccharides, glycoproteins, carbohydrate-protein matrix or protein and polysaccharide combinations (Arndt, 1960a,b; Stahl and Leray, 1961; Guraya, 1965, 1978; Anderson, 1967; Nakano, 1969; Tesoriero, 1977b; Makeeva and Mikodina, 1977; Pelizaro *et al.*, 1981, Lopes *et al.*, 1982; Stehr, 1982). Khoo (1979) detected phospholipids in the zona radiata of gold fish oocytes in addition to carbohydrates, proteins and amino groups. Results of the present study are in conformity with these observations.

The histochemical characteristics of zona radiata externa and zona radiata interna have shown some perceptible differences in the oocytes of *S. sihama*. Carbohydrates were more abundant in the zona radiata externa than in the inner layer. Only zona radiata externa contained lipids and this layer contained relatively more RNA than zona radiata interna. Other workers have also reported difference in the histochemical properties of various layers of zona radiata. Nakano (1969) could not detect carbohydrates in the zona pellucida proper, but only a thin layer outside the zona pellucida contained polysaccharides. In the sea bass, *Dicentrarchus labrax*, only the zona radiata externa stained for carbohydrates (Mayer *et al.*, 1988). In teleosts, such as *Cichlosoma nigrofasciata* (Busson-Mabillot, 1977), *Salmo gairdneri* (Hagenmaier, 1973) and *Noemachielus barbatulus* (Riehl, 1978c), the inner layer of zona pellucida consists only of protein, while the outer layer a combination of protein and polysaccharides. Such variations in the zona material, though known, little is understood regarding their significance. Further studies on the molecular organisation of different layers of zona radiata are necessitated for better understanding of their functional roles in the biology of fish eggs.

Yolk vesicles

The beginning of vitellogenesis is marked by the appearance of cortical alveoli (Yolk vesicles) and yolk bodies in the oocyte (Raven, 1961; Kraft and Peters, 1963; Malone and Hisaoka, 1963; Guraya, 1965, 1982, 1986). The rapid growth of oocyte is due mainly to the accumulation of the cortical alveoli and yolk. This phase is generally called vitellogenesis, which is accompanied by intense metabolic activity (Nakano, 1969) and is regulated by hormones. The cortical alveoli are now believed to be synthesised endogenously (te Heesen, 1977; Mayer *et al.*, 1988).

Guraya (1965), using histochemical techniques, suggested that the cortical alveoli in *Channa marulius* first appear near the plasma membrane by means of pinocytotic activity. However, a careful examination of growing oocytes of *C. punctatus* treated with PAS-technique, has revealed that small PAS-positive granules first originate in the outer ooplasm (Guraya, 1982). The results of the present work are in agreement with this observation. PAS-positive granules made their appearance in the outer ooplasm of primary yolk granule oocyte stage in *S. sihama*.

Extensive cytochemical studies have been carried out of the cortical alveoli in the oocytes of different teleost species (Guraya, 1986, for review). Glycogen is reported to be absent in the cortical alveoli of several fishes Yamamoto, 1955a, 1956b-d; Rajalakshmi, 1966; Gopal Dutt and Govindan, 1969). But in other fishes, glycogen is said to be present in the cortical alveoli (Krishnamurthy *et al.*, 1972; Shahi *et al.*, 1979; Ramadan, 1979b). In the present study, staining for glycogen was very weak in the yolk vesicles. This is supported by the biochemical data furnished in Chapter VI, where it was shown that ovary contains relatively low glycogen content which tends to decrease in the final stages of maturation.

It is widely accepted that PAS-positive substances (carbohydrates) form the most characteristic feature of cortical alveoli during their development and maturation (Guraya, 1986). Yolk vesicles of *S. sihama* oocytes were highly PAS-positive and staining after taka diastase treatment removed the dye, confirming their carbohydrate nature.

Yamamoto (1956e) believes that according to the habitat, the type of polysaccharide may vary in fish eggs. In marine teleosts, such as *Clupea pallasii* and *Liopsetta obscura*, only neutral mucopolysaccharides were detected. This is in agreement with the present results that acid mucopolysaccharides could not be detected in *S. sihama* oocytes. Acid mucopolysaccharides are abundant in freshwater teleost eggs, while in anadromous species like *Hypomesus japonicus* during the development, first netural and later acid mucopolysaccharides were detected (Yamamoto, 1956e).

Proteins are reported to be present in the yolk vesicles (Anderson, 1968; Shahi *et al.*, 1979; Khoo, 1979), while some workers have observed a carbohydrate-protein complex in the same (Krisnamurthy *et al.*, 1972, Donato 1980; Guraya, 1982). Khoo (1979) showed high concentration of sulphydryl groups in the yolk vesicles of gold fish oocyte. This observation is in conformity with the present results. In *S. sihama* oocytes, yolk vesicles stained weakly for general proteins, while strongly to -SH groups. RNA and lipids were absent in the yolk vesicles. Guraya (1986) also opines that sudanophilic lipids are not present in the cortical alveoli of fish oocytes at any stage of their development and maturation.

The cortical alveoli are believed to undergo 'cortical reaction' at fertilization (Guraya, 1982). In the present work, the yolk vesicles were seen close to the zona radiata in the tertiary yolk granule oocyte. These vesicles are believed to release their contents into the perivitelline space between the zona radiata and the protoplasmic surface. This in turn, is supposed to lead to the formation of the perivitelline space which widens rapidly to block polyspermy. However, the actual process of 'cortical reaction' could not be observed in the present study.

With the advancement of maturation, yolk accumulates in the platelets, resulting in considerable increase in ovarian weight (Wiegand, 1982). Traditionally yolk has been classified by histochemical methods as carbohydrate, lipid and protein yolk (Raven, 1961; Guraya, 1965). The yolk vesicles, rich in carbohydrate material are the likely precursors of cortical alveoli, which are extruded at the time of fertilization and hence should not be considered as true yolk (Wiegand, 1982). It is, thus more reasonable to state that the yolk bodies in fish eggs consist chiefly of two components, namely fatty (lipid) yolk droplets and proteid yolk granules or globules. During the terminal stages of oocyte maturation, the egg yolk generally coalesces and mixes with the other egg contents (Yamamoto and Oota, 1967a,b; Goetz, 1983).

It is generally agreed that yolk is formed in fish oocytes by either of the three methods, such as endogenous vitellogenesis (oocyte organelles contribute to yolk formation), exogenous vitellogenesis (yolk precursor is fabricated in liver and sequestered in the ooplasm by micropinocytosis) or a combination of both (Guraya, 1986).

Fatty yolk

The mode of formation of oil droplets during vitellogenesis varies considerably. Oil droplets appear earlier than the yolk vesicles in some fish and in some others they appear simultaneously with the yolk vesicles (Ishida, 1980). Generally lipid deposition occurs prior to that of the proteid yolk (Raven, 1961). In the oocytes of *S. sihama*, Oil droplets made their appearance prior to that of yolk vesicles and yolk granules. Its appearance can be considered to be the beginning of endogenous vitellogenesis (Shackley and King, 1977). Wiegand (1982) has suggested that since the major yolk protein precursor, Vitellogenin, and its derivative lipovitellin contain lipid, it should be remembered that the classicial term 'lipid yolk' is normally reserved for lipid inclusions observed in the anatomical studies to be distinct from vitellogenin-derived yolk granules. Oocytes of some fish species do not contain lipid yolk (Khoo, 1979).

Fatty yolk in fish eggs consists mainly of neutral fats (Raven, 1961; Chopra, 1958a,b, 1960; Guraya, 1965; Ramadan *et al.*,1979b). In the present material, the oil droplets were found to contain neutral lipids and phospholipids, respectively, in high and moderate concentrations. The oil droplets appeared in the form of small sudanophilic granules around the nucleus in the vacuolated oocyte stage. With further growth of the oocyte, these granules coalesced to form large oil droplets. In the tertiary yolk granule oocyte stage, the oil droplets were very large and spread almost throughout the ground cytoplasm. Droller and Roth (1966) have observed the coalescence of small lipid granules with larger droplets at the ultrastructural level in the oocytes of guppy. Goetz (1983) has discussed the species variations in regard to the degree of coalescence or fusion of fatty yolk globules.

Protein yolk

In the oocytes of *S. sihama*, the protein yolk (yolk granules) accumulation occurred after lipid yolk accumulation, in the cortical regions of the oocyte and gradually extended centripetally, to fill the ooplasm. The protein yolk was devoid of carbohydrate material. But in several freshwater fishes, the proteid yolk contained polysaccharides in addition to the protein and lipid components(Guraya, 1965; Verma, 1977; Riehl, 1977). It is generally shown that proteid yolk in fish eggs consists, chiefly of protein and lipoproteins (Chopra, 1958a,b, 1960; Malone and Hisaoka, 1963; Lal, 1963; Guraya, 1965; Rastogi, 1969; Khoo, 1979; Shahi *et al.*, 1979).

In the oocytes of *S. sihama*, the yolk granules contained high concentration of general proteins, amino groups, S-S groups, tyrosine and tryptophan, while -SH groups were detected only in traces. The yolk granules also moderately responded to the staining for lipids and RNA. The RNA content of yolk granules may have been a contribution from nucleoli. In *Paratelphus* and *Dysdercus*, Nath *et al.*, (1929)(as quoted by Saxena *et al.*,1979) have found direct transformation of nucleolar material into the protein yolk granules. Similar observations were made in *Gambusia affinis* (Verma, 1977) and *Mastacembelus armatus* (Saxena *et al., 1979)*. The present observation that -SH groups are poorly represented in the yolk granules, is in conformity with the results obtained in gold fish (Khoo, 1979).

It is now know that exogenous vitellogenesis involves in the synthesis of vitellogenin, the major yolk precursor, in the liver under the influence of oestrogens and carried through the blood plasma, to be sequestered in the ooplasm by

micropinocytosis (Wallace, 1978; Wallace and Selman, 1981; Ng and Idler,1983). The micropinocytotic activity at the surface of vitellogenic oocytes during the time of yolk deposition has been demonstrated by electron microscopy for several teleost fishes (Droller and Roth, 1966; Anderson, 1968; Wegmann and Gotting, 1971; Upadhyay, 1977; Updhyay *et al.*, 1978; Selman and Wallace, 1982a, Ng and Idler, 1983). In the present work, zona radiata interna of the yolk granule oocytes (mostly tertiary stage) showed convoluted nature, which are indicative of the micropinocytotic activity at the oocyte surface.

Germinal vesicle

With the initiation of oocyte growth, the chromosomes undergo distinct morphological and histochemical changes. In the present work, chromatin DNA content was detectable in the primary oocyte stage. But the staining for DNA became weak in the vacuolated oocyte stage and DNA was not detected in the yolk granule oocyte stage. This result is in conformity with that of other works (Brachet, 1960a; Hess and Meyer, 1963; Kraft and Peters, 1963; Rastogi, 1968a; Saxena *et al.*,1979). Brachet (1960) offers explanation that DNA in late oocytes may be too dilute in the nucleoplasm to show a visible colouration with Schiff's reagent. Hess and Meyer (1963) believe that the negative results of the Feulgen reaction for DNA may be due to the largely spiralized nature of the lampbrush chromosomes, which make their appearance during the diplotene stage. In some fishes DNA was not detected at any stage of maturity of the oocyte (Marza *et al.*, 1937; Hisaoka and Firlit, 1962; Verma, 1977).

Nucleoplasm and nuclear membrane of the oocytes of *S. sihama* were found to contain only traces of proteins. RNA content of the nucleoplasm

tended to decrease as the maturation advanced. RNA was detected in moderate quantity in the primary oocyte, but only in traces in vacuolated and yolk granule oocytes. Korfsmeier's (1966) finding in the zebra fish, *Brachydanio rerio* that high rate of RNA synthesis occurs only in the previtellogenic oocytes, thus, is in agreement with the present results. However, Guraya (1965) observed high concentration of RNA in the nucleoplasm and ooplasm of mid-vitellogenic oocytes of teleost fish.

Various histochemical studies have revealed the presence of some intranuclear lipid bodies in the previtellogenic oocytes of teleosts (Subramaniam and Aiyar, 1935; Seshachar and Nayyar, 1963; Guraya, 1965; Gopal Dutt and Govindan, 1967; Saxena and Bhatia, 1977; Ramadan *et al.*,1979b). In the present study also, moderately Sudan B-positive granules were found in the nuclei of primary oocyte and Vacuolated oocyte stages, which were distinct from nucleoli. They were absent in the yolk granule oocytes. The contents of the intranuclear lipid bodies seem to be diffusing outside the nucleus to contribute new lipid bodies, which again originate in the form of granules (Guraya, 1986). However, it was not possible to trace the fate of the intranuclear lipid bodies in the present material. Ramadan *et al*,(1979b), working on the young oocytes of *Merluccius merluccius*, have observed these lipid bodies, which finally passed into the ooplasm to play some role in yolk formation.

Cytochemical techniques have demonstrated RNA and proteins in the nucleoli of fish oocytes (Guraya, 1963, 1965). In *S. sihama*, nucleoli of the oocytes showed increase in intensity of staining for proteins from the primary oocytes to vacuolated oocytes. General proteins and amino groups

were present in appreciable amounts. S-S groups tended to become less and -SH groups more with the advancement of maturation. Nucleoli also contained high concentration of RNA. Caspersson (1950) and Brachet (1960a) have advocated the hypothesis that the high concentration of RNA in the nucleoli is concerned with protein synthesis.

Nucleolus is identified as an important organelle from the point of view of biogenesis of cytoplasmic ribosomes (MacGregor, 1972; Wischnitzer, 1973; Davidson, 1976). Ribosomal RNAs formed in the nucleolar apparatus, apparently by way of precursor molecules (Roger, 1968; MacGregor, 1972; Wischnitzer, 1973; Davidson, 1976), need transport mechanisms by which material of this type may reach the cytoplasm. Several workers have observed a phenomenon called 'nucleolar extrusions', which are believed to contribute precursors for ribosomes to the ooplasm (Raven, 1961; Guraya, 1965; Guraya *et al.*, 1975; Verma, 1977; Saxena *et a.*, 1979; Srivastava and Swarup, 1979; Ramadan *et al.*, 1978; Shrestha,1980). The nucleolus appears to pass into the cytoplasm, breaks up into pieces and develops lipoproteins and some lipid granules in addition to its original RNA and proteins (Guraya, 1965).

In the present study too, 'nucleolar extrusions' were observed. In TMOG staining, the peri-nuclear area of the vacuolated oocytes appeared deep blue, indicating abundance of RNA in this region. This could have resulted from 'nucleolar extrusions'. Further, in routine histology also, it was seen that the nucleoli tended to lie partly inside and partly outside the nuclear membrane in the previtellogenic oocytes, which are likely to pass into the ooplasm through the nuclear envelope. Kapoor (1977) found that some of the nucleoli lying in

contact with the nuclear envelope, are passed into the surrounding cytoplasm of oocytes in *Puntius ticto* and finally disappear near the periphery of the oocytes. In the present material, highly RNA-positive granules were found lying in the ooplasm in yolk granule oocytes, which might be pieces of nucleoli extruded from the nucleus. Observations of Raikova (1976) in acipenserid fishes and that of Saxena and Bhatia (1977) in *Heteropneustes fossilis* support this view point.

The results of the histochemical studies of oocytes of *S. sihama* during their growth and maturation are in general conformity with those of the biochemical changes of the whole ovary discussed in Chapter VI. The eggs supply both genetic material and nutrients for the development of embryo, which accounts for the translocation of the biochemical components from the body parts to the ovaries. The changes in carbohydrate, protein, lipid and nucleic acids of the oocyte constituents are reflective of the formation of yolk during vitellogenesis.

CHAPTER VIII

PRELIMINARY EXPERIMENTS OF INDUCED MATURATION AND SPAWNING

Breeding is one of the most important facets of fish culture practices. By the manipulation of environmental factors, such as temperature, photoperiod etc and by using hormones, such as fish pituitary gland extract and mammalian hormones, fish are bred in capacity. The successful application of hypophysation techniques in the spawning of fishes is considered a land mark in fish culture. Houssay (1930, 1931) was the pioneer in the field of hormonal control of reproduction of fish. The technique of induced breeding of fish was soon applied in Brazil (Von Ihering 1935, 1937; Von Ihering and Azevedo, 1934). Gerbil'skii (1938) succeeded with the induced spawning of sturgeon in USSR, and Hasler (1939, 1940) in the United States. These successes set the stage for more and more workers to try and develop suitable induced spawning techniques in several teleost species.

The present status of knowledge on the uses of different hormones in fish culture, role of nutrition and environmental factors in brood stock management, cryopreservation of gametes as well as advanced techniques, such as chromosome manipulation were the objects of a number of reviews (Pickford and Atz, 1957; Atz and Pickford, 1959; Clemens and Sneed, 1962; Das and Khan, 1962; Atz and Pickford, 1964; Chaudhuri, 1968, 1969; Clemens, 1968; Jhingran, 1969; Ibrahim, 1969; Shehadeh, 1973; Donaldson, 1973, 1975; Fontaine, 1976; Harvey and Hoar, 1979; Chondar, 1980; Woynarovich and Horvath, 1980; Pullin and Kuo, 1981; Davy and Chouinard, 1981; Sundaraj, 1981; Lam, 1982; Fostier and Jalabert, 1982; Donaldson and Hunter, 1983).

Most of the fish species cultured at present are freshwater fishes and high rates of success have been reported in the induced breeding of many of them (Pullin and Kuo, 1981). Among the brackishwater teleosts, the technical feasibility of artificial propagation and culture has been proved in several species of mullets, especially *Mugil cephalus* (Lee and Tamaru, 1988). There is a large number of marine species which could be considered potential candidates for culture and the reports on induced spawning and larval rearing successes are continuing to increase (Pullin and Kuo, 1981).

Literature on the artificial propagation of sillaginid fishes is scanty and most of the works have been carried out in Japan. Spawning of *Sillago japonica* (= *S. sihama*) collected from the wild and that produced in the laboratory has been reported (Kumai and Nakamura, 1977, 1978). The same species was induced to spawn by injecting hormone Puberogen as well as by manipulating water temperature and photoperiod (Lee, 1979, 1981 a & b,1985). Kashiwagi *et al.* (1984) described the laboratory spawning of *S. japonica*.

The only previous report on the induced breeding experiments on *Sillago sihama* from India is that of James (1984). During the present investigation, a series of experiments were conducted on the induced maturation and spawning of *S.sihama* collected from Cochin backwaters using major carp pituitary extract and human chorionic gonadotropin. These experiments were preliminary in nature and were carried out for a brief period of one month.

MATERIALS AND METHODS

Experiments on induced maturation on spawning of *Sillago sihama* were carried out only for a brief period from October to November, 1985.

Fish was collected form Cochin backwaters near Vypeen island (Plate XL). The fish is caught in dip nets planted along the shore. The net, locally known as 'chinese dip net', catches those fishes moving along the coast during flood and ebb tides. It was observed that *Sillago* is caught in good quantity only during July to November in this gear. The species is rarely caught in chinese dip nets during other months.

Fishes were taken from the main net by a scoop net and transferred to 50 L plastic basins containing water taken from the same region in which the dip net was operated. The basins containing live fishes were transported to the Narakkal Prawn Culture Laboratory at Narakkal, where the experiments were conducted. There was no mortality during transportation.

The water in which fish were transported from the collection site had salinity ranging from 14 to 17 ppt. In the laboratory, fish were gradually acclimated to higher salinity varying between 25 and 27 ppt.

The fishes were maintained in 3' dia polycraft pools and 100 L perpex tanks and water was aerated (Plate XLI, Fig. 1-3). Initially many fishes were found infected with bacteria after maintaining them for 2 - 3 days in these pools. To overcome this problem, before putting them in the pools, the fishes were given a dip in Potassium permanganate solution. In addition, Pencillin (100 ppm) was added to the water contained in the pools. These measures proved effective in checking bacterial infection. Each pool contained 2 - 3

PLATE XL.

Map showing the area of collection of *S. sihama* for the induced maturation experiments.

PLATE XL

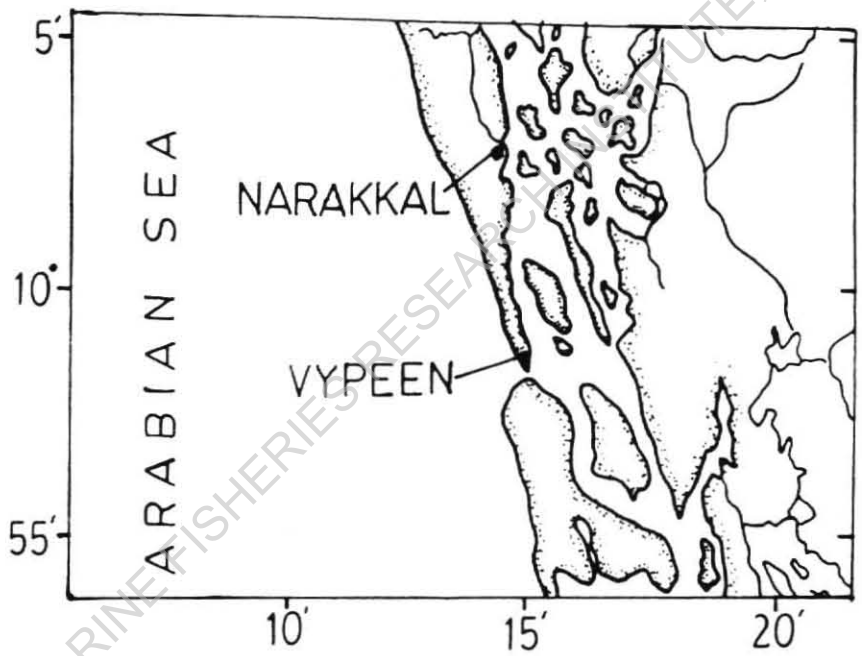


PLATE XLI

Fig.1. Laboratory set-up for conducting induced maturation and spawning experiments.

Fig.2 & 3. Perspex tanks containing live *S. sihama*.

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fishes at a time. No feeding was done during the experiments.

Since there were no characters by which sex could be differentiated externally, identification of sex was done by examining the reproductive elements using a canula (Shehadeh *et al.*,1973b). This method was also useful for accurate determination of the maturity stage of the female fish.

A plastic canula of 0.8 mm diameter was introduced into the oviduct through cloaca (Plate XLII, Fig. I). Slowly ova were withdrawn into the canula by sucking orally as the tube was withdrawn. Ova were drawn from the mid portion of the ovary as they were the most representative.

The ova were immediately placed on a slide and diameter measured under the microscope with an ocular micrometer at a magnification which gave a value of 15.38 μm to each micrometer division.

Before injecting the fish, total length (in mm) and body weight (in g nearest to 0.1g) were taken. Body weight was taken by the following method; First the fish was wrapped in a polythene bag and weight was taken. Then weight of the poythene bag alone was taken. The difference between the two weights gave the body weight of fish.

Body weight of the fishes subjected to induced maturation and spawning experiments ranged from 55 to 250 g in female fish and from 45 to 100 g in male fish. Majority of the female fishes, before giving injections were in stage II (Maturing) with the largest clutch of ova measuring 0.32 mm in diameter and showing signs of yolk accumulation. A few were in stage III (Mature), wth the largest clutch of ova measuring 0.47 mm in diameter and showing signs of advanced yolk accumulation. The three male fishes subjected to this study,

were not in mature condition, as evidenced by the fact that when pressed along the abdomen, no milt oozed out.

Injections of hormones were given intramuscularly (Plate XLII, Fig.2) using sterilized hypodermic syrings (GLASS WAN- 1,2 and 5 ml) and needles of 19 and 21 gauges.

Major carp pituitary extract (West Bengal state Fishermen's Co operative Federation Ltd) and human chorionic gonadotropin (Profasy Serono-Rome and Pubergen CG) were the hormones used during the present study. Different dosages tried were: HCG - 800 IU, 1000 IU, 2000 IU, 3000 IU and 5000 IU. Carp pituitary gland - 60 mg, 100 mg, 120 mg and 150 mg (all per kg body weight of the fish).

While injecting, the head of the fish was covered with a conical opaque plastic bag, to prevent the fish from getting disturbed. The injections were given during the period between 16:00 and 20:00 hours.

The injected fish were examined for any change in their gonadal development, 24/48h after the injection. This examination was done by canula method (female fish) and by testing milt ejaculation (male fish). In fully mature female, the belly was rounded and bulged. The medium was also closely examined to see whether any eggs were released in it.

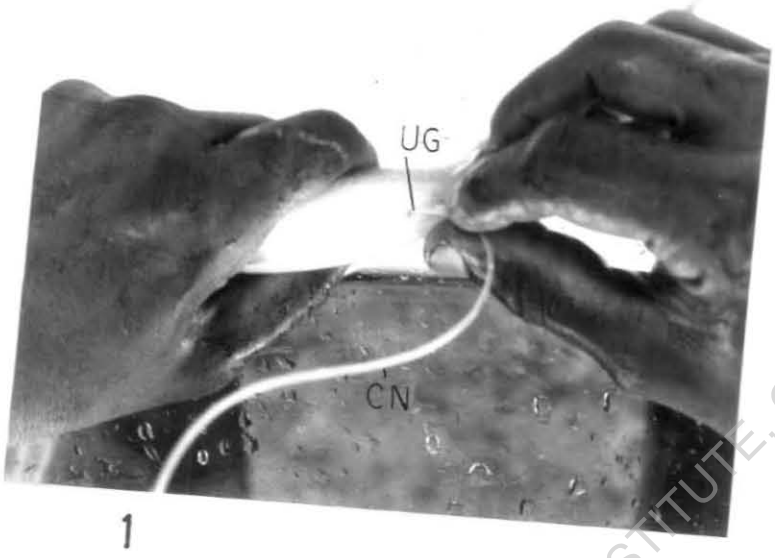
Whenever the fishes were found in ripe condition, they were removed from the pool and stripped. The spermatozoa and ova were mixed by 'dry method' for fertilization. The mixed reproductive elements were kept in an aquarium tank containing water having salinity ranging between 25 and 27 ppt. The medium was well aerated and temperature was maintained between 26 and 27 degree C.

PLATE XLII

Fig.1. Withdrawal of intra-ovarian eggs through a plastic canula. CN = Canula; UG = Urinogenital opening

Fig.2. Intramuscular injection of hormones.

PLATE XLII



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OBSERVATIONS

Experiments with major carp pituitary gland extract

On six occasions, carp pituitary gland extract was given as priming dose and once as booster dose (Table 42). Three females in stage II (the most advanced clutch of oocytes with a mean diameter of 0.32 mm and in secondary yolk granule stage), weighing 120, 100 and 75 g. received doses of 100, 150 and 120 mg/Kg body weight of the pituitary gland extract, respectively. The ovary of the fish injected with 100 mg/Kg body weight of the hormone, developed to stage IV, but the fish died without spawning. The fish was found heavily infected with bacteria. The ovary of the fish which received 150 mg/Kg body weight of the pituitary gland extract developed to stage IV within 48 hours and the fish spawned. The eggs had a mean diameter of 0.67 mm and contained multiple oil globules. These eggs were fertilized by mixing them with milt taken from a mature male. The fertilized eggs contained clear perivitelline space and germinal disc. However, they failed to develop further and degenerated. The fish which was given 120 mg/Kg body weight of the extract developed to stage III within 24 hours.

A female in stage III (the most advanced clutch of oocytes with a mean diameter of 0.47 mm and in tertiary yolk granule stage) and weighting 250 g, on receiving 60 mg/Kg body weight of the pituitary gland extract, advanced in maturity to stage IV, but died without spawning. Heavy infection by bacteria in the caudal peduncle region was noticed. Another female in stage III and weighing 67.5 g, after injected with 150 mg/Kg body weight of the extract, developed to stage IV and spawned within 48 hours. In this case too, the eggs contained multiple oil globules, and though fertilized, did not develop further.

TABLE 42. Response to carp pituitary gland extract

S.No.	Sex	Weight (g)	Dosage (per kg body wt)	Nature of injection	Stage of maturity	Mean diameter and stage of development of the most advanced clutch of ova	Timing Injection / Examination	Response & Remarks
1.	Female	120	100 mg	Priming Dose	II	0.32 mm Secondary yolk granule stage.	4-10-1985 at 1700h / 5-10-1985 at 1600h	Ovary developed to Stage IV. No spawning. Fish dead. Heavy infection of fish noticed.
2.	Female	100	120 mg	Booster Dose	III	0.47 mm Tertiary yolk granule stage	6-10-1985 at 1645h / 7-10-1985 at 1600h	No development on 9.10.85 at 1600h. The ovary was in immature condition. Fish heavily infected.
3.	Female	250	60 mg	Priming Dose	III	0.47 mm Tertiary yolk granule stage	7-10-1985 at 1715h / 8-10-1985 at 1630h	Developed to stage IV. Fish dead without spawning. Heavy infection of fish.
4.	Female	100	150 mg	Priming Dose	II	0.32 mm Secondary yolk granule stage	7-10-1985 at 1730h / 9-10-1985 at 1600h and 10-10-1985 at 1700h	On 9th fish was in stage IV and on 10th spawning was observed. Eggs with multiple oil globules. Eggs fertilized, but no further development.

contd.....

TABLE 42. (Contd.....)

S.No.	Sex	Weight (g)	Dosage (per kg body wt)	Nature of injection	Stage of maturity	Mean diameter and stage of development of the most advanced clutch of ova	Timing Injection / Examination	Response & Remarks
5	Female	75	120 mg	Priming Dose	II	0.32 mm Secondary yolk granule stage	18-10-1985 at 1650h / 19-10-1985 at 1700h	Developed to stage III
6	Male	45	280 mg	Priming Dose	'Non-semin-ating'	-	18-11-1985 at 2000h / 20-11-1985 at 2095h	Became mature and milt oozed out freely on 20th.
7	Female	67.5	150 mg	Priming Dose	III	0.47 mm Tertiary yolk granule stage	21-11-1985 at 1600h / 23-11-1985 at 1600h	Fish spawned. Diameter of ova 0.617-0.73 mm. Multiple oil globules in the ova. Eggs fertilized, but no further development.

120 mg/Kg body weight of pituitary gland extract was given as a booster dose to a female in stage III, weighing 100 g. There was no further gonadal development but the ovary regressed to immature condition within 24 hours of injection.

One male, weighing 45 g and in 'non-seminating' condition advanced in maturity to stage IV (Oozing stage) within 48 hours of receiving 280 mg/Kg body weight of the pituitary gland extract.

Experiments with human chorionic gonadotropin (HCG)

On seven occasions, HCG was given as priming dose and twice as booster dose. HCG-Profasy was administered on six occasions and HCG-Pubergen once as priming dose, while they were given once each as booster dose (Table 43).

Four females weighing 125, 110, 150 and 55 g received HCG-Profasy in doses 800, 5000, 3000 and 4500 IU/Kg body weight, respectively. One female, weighing 145 g was injected with 3000 IU/Kg body weight of HCG-Pubergen. The fish which received 800 IU/Kg body weight of the hormone did not show ovarian development within 24 hours of injection and was found heavily infected with bacteria on the caudal peduncle. The fish which received 5000 IU/Kg body weight of HCG, showed advancement of maturity to stage IV from stage II within 24 hours. Of two females weighing almost the same (145 and 150 g) and in the same maturity stage (stage II), the one which was given 300 IU/Kg body weight of HCG-Pubergen showed gonadal development to stage IV, where as the one which received the same dose of HCG-Profasy did not show any further development in maturity and died. The fish, weighing

TABLE 43. Response to human chorionic gonadotropin and a combination of pituitary gland extract and human chorionic gonadotropin

S.No.	Sex	Weight (g)	Dosage (per kg body wt)	Nature of injection	Stage of maturity	Mean diameter and stage of development of the most advanced clutch of ova	Timing Injection / Examination	Response & Remarks
1.	Female	125	800 (HCG-profasy)	Priming Dose	II	0.32 mm Secondary yolk granule stage	4-10-1985 at 1730h / 5-10-1985 at 1800h	No development in the ovary. Heavy infection of fish.
2.	Male	100	1000 (HCG-profasy)	Priming Dose	Non-semin-ating	-	4-10-1985 at 1750h / 6-10-1085 at 1700h	Became mature and milt oozed and on 6th.
3.	Male	60	2000 (HCG-profasy)	Priming Dose	-do-	-	4-10-1985 at 1750h / 6-10-1985 at 1730h	Became mature and milt oozed out on 7th.
4.	Female	110	5000 HCG-Profasy	Priming Dose	II	0.32 mm Secondary yolk granule stage	7-10-1985 at 1730h / 8-10-1985 at 1700h	Developed to stage IV. Fish dead without spawning. Heavy infection of fish.
			500 0 (HCG-pubergen)	Booster Dose	IV	0.65 mm Tertiary yolk granule stage and hyaline stage	8-10-1985 at 1945h / 9-10-1985 at 1645h	No Spawning on 9th. Atresia was noticed.

(Contd.....2.)

TABLE 43. (Contd.....)

S.No.	Sex	Weight (g)	Dosage (per kg body wt)	Nature of injection	Stage of maturity	Mean diameter and stage of development of the most advanced clutch of ova	Timing Injection / Examination	Response & Remarks
5.	Female	145	3000 (HCG-pubergen)	Priming Dose	II	0.32 mm Secondary yolk granule stage	8-10-1985 at 1640h / 10-10-1985 at 1700h	Developed to stage IV. stripped on 10th. No fertilization.
6.	Female	150	3000 HCG-profasy)	Priming Dose	II	0.32 mm Secondary yolk granule stage	9-10-1985 at 1830h / 10-10-1985 at 17304	No development. Fish died on 10th.
7.	Female	75	3000 HCG-profasy)	Booster Dose	III	0.47 mm Tertiary yolk granule stage	19-10-1985 at 1645h / 20-10-1985 at 1700h	Developed to stage IV and spawned on 20th. Eggs fertilized but no further development.
8.	Female	55	4500 HCG-profasy)	Priming Dose	II	0.32 mm Secondary yolk granule stage	21-11-1985 at 1600h / 22-11-1985 at 1700h	Ovary found regressed to immature condition.
9.	Female	100	1000 I U HCG + (profasy) 60mg Carp pituit-ary gland extract	Priming Dose	II	0.32 mm Secondary yolk granule stage	4-10-1985 at 1745h / 5-10-1985 at 1700h	Developed to stage III on 5th.

55 g and in stage II, on being injected with 4500 IU/Kg body weight of the hormone, exhibited regressive development of the ovary.

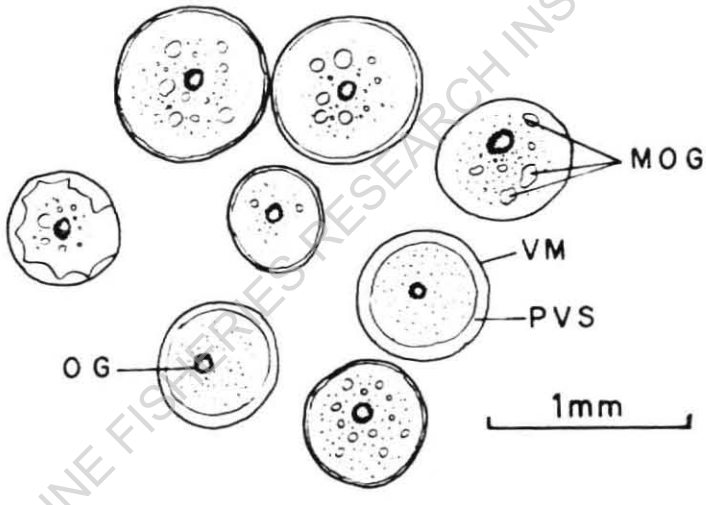
When 5000 IU/Kg body weight of HCG-Pubergen was administered as booster dose to the female, which has already developed to stage IV from stage II on receiving 5000 IU/Kg body weight of HCG-Profasy (priming dose), did not spawn and the intraovarian eggs were mostly atretic. A female weighing 75 g and in stage III was injected with 3000 IU/Kg body weight of HCG-Profasy as booster dose (this fish had received 120 mg/Kg body weight of carp pituitary gland extract as priming dose) and the fish spawned. The eggs had a mean diameter of 0.66 mm and most of them contained multiple oil globules (Plate XLIII). Eggs, though could be fertilized, did not develop further.

Two males weighing 100 and 60 g were given 1000 and 2000 IU/Kg body weight of HCG-Profasy, respectively. Both these specimens were in 'non-seminating' condition prior to the administration of the hormone and their testes developed to stage IV within 48 hours of injection.

Experiment with combination of major carp pituitary gland extract and HCG

One female in stage II and weighing 100 g was given the combined dose (60 mg pituitary gland extract + 1000 IU HCG-Profasy per Kg body weight) as priming dose (Table 43). The ovary advanced in maturation to stage III within 24 hours of injection. This fish, though had received a booster dose of 120 mg/Kg body weight of pituitary gland extract, did not show further development of its gonads.

PLATE XLIII



REMARKS

Brood stock management and induced breeding are very important aspects of aquaculture and excellent reviews on them have been provided by Shehadeh (1973) and Pullin and Kuo (1981). The experimental work reported here is of a preliminary nature and is an attempt to see how *Sillago sihama* responds to various hormone treatments to induce gonadal maturation and spawning.

The use of pituitary preparations to induce maturation and spawning in teleosts has a long and successful history. Mammalian hormones are known to have more advantages than the pituitary preparations, like more easy availability, greater uniformity and also could replaced the tedious procedure of collection, preserving, processing and distributing pituitary material (Shehadeh, 1973).

In the present study, major carp pituitary gland extract and human chorionic gonadotropin (HGG) were used to induce gonadal maturation. Stage of maturity of ovary was found to advance on receiving 60, 100, 120 and 150 mg/kg body weight of pituitary gland extract given as priming doses. Two females in stages II and III, weighing 100.0 and 67.5 g, spawned within 72 and 48 hours, respectively, after being injected with 150 mg/kg body weight of pituitary extract. A male fish, weighing 45 g and in 'non-seminating' condition, developed to maturity on receiving 280 mg/Kg weight of pituitary extract.

In the series of trials conducted with HCG, ovarian maturity was found to advance by doses of 3000 and 5000 IU/Kg body weight given

as priming doses. A female fish, weighing 75 g, on receiving 3000 IU/Kg body weight of HCG as booster dose, spawned. This particular fish was earlier given 120 mg/Kg body weight of pituitary gland extract as priming dose. Like the pituitary gland extract, HCG also proved effective in inducing the testicular maturation, as shown by the development of 'non-seminating' testes of two fishes to 'Oozing' stage by administering 1000 an 2000 IU/Kg body weight of HCG. Injection with a combination of pituitary gland extract and HCG induced advancement of maturation in a female.

The spawned eggs of *S.sihama* could be fertilized, but there was no further development. Majority of the eggs contained multiple oil globules. In *Mugil cephalus*, Kuo *et al.* (1973a) observed that multiple oil globules were noticed only when eggs were removed from the gravid females by manual pressure. These workers have inferred that oil droplets of the oocytes may be directly related to premature inducement by manual extrusion or to incomplete inducement following hormonal injection. Such formed eggs, as in the present work, were fertilized, but failed to divide. Spontaneous release of eggs by the female produces eggs with a single oil globule, which are invariably fertilized and develop naturally, producing viable larvae (Nash and Kuo, 1975).

In *Euthynnus affinis*, Kaya *et al.* (1981) have attributed the poor viability of eggs to the failure to detect or respond to induced ovulation by hormones early enough, with a consequent deterioration of the ovulated eggs within the ovarian lumen. The phenomenon of 'overripening' has been described by Nomura *et al.* (1974). Sakai *et al.* (1975) showed that the viability of *Salmo gairdneri* ova left unstripped in the female after

decreased very rapidly. A smilar observation was made in ayu, *Plecoglossus altivelis* also (Hirose *et al.*, 1977). In the present study too, it appears that majority of the eggs would have undergone deterioration before being liberated by stripping.

In Japan, *Sillago japonica* spawners were reported to spawn on receiving 330 IU Puberogen (Lee *et al.*, 1981a). Lee and Hirano (1985) have reported spawning of the same species by manipulating water temperature and photoperiod. They found that fish acclimated to the warmer water and normal photoperiod matured earlier than those in cold water and that the fish started spawning as water temperature rose over 20' C (68'F). In India, induced breeding of *Sillago sihama* was attempted at Mulky, near Mangalore (James, 1984). He found that the fish responded positively to the injections of catfish pituitary gland extracts and one female spawned in an experimental plastic pool. But attempts to fertilize the eggs were unsuccessful.

SUMMARY

1. The thesis presents a comprehensive account of reproduction in *Sillago Sihama* (Forskal) collected from selected landing centres of the Palk Bay and Gulf of Mannar, together with a systematic study of the sillaginids from this region and some preliminary experiments on the induced maturation and spawning of *S. sihama* collected from Cochin backwaters.

2. Following the review of literature on distribution, fisheries, systematics, biology and culture of sillaginids and Materials and Methods of investigation, the study begins with taxonomic considerations of the family sillaginidae.

3. Because of their close external similarities, several species of sillaginid fishes are usually misidentified. Based mainly on the structure of swimbladder, six species of the family Sillaginidae are identified from Palk Bay and Gulf of Mannar. *Sillago sihama*, the most dominant species of the family, constitutes about 60% of all sillaginid species landed in commercial catches in the present area of investigation. The other 5 species in the order of abundance are *Sillago indica*, *S. argentifasciata*, *S. soringa*, *S. vincenti* and *S. chondropus*.

4. The female reproductive system consists of a pair of ovaries, oviducts and a common ovarian duct that opens out at the genital pore and the male reproductive system consists of a pair of testes, vasa deferentia and a common sperm duct which also opens at the genital pore. Based on macroscopical and microscopical observations, 5 stages of maturation, namely immature, maturing, mature, ripe and partially spent stages have been identified in female. Based on the external characteristics of the

testes, maturity of male has been classified into 5 stages, namely immature, maturing, mature, oozing and partially spent.

5. Results of the ova diameter studies indicate that each individual female spawns more than once. Studies on the monthly occurrence of different maturity stages of both sexes and variations in monthly mean gonadosomatic index values reveal that *S. sihama* has a prolonged breeding season, extending from about July to February, with peak spawning activity during July to November period.

6. Minimum size (total length) at first maturity of female is 179 mm and that of male 159 mm. Fecundity varies from 6956 to 48,373 in individuals of total length ranging between 150 and 210 mm. Fecundity is curvilinearly related with total length (Log F = -8.1812 + 5.5458 LogL) and linearly with body weight (Log F = 1.4169 + 1.7418 LogW) and ovary weight (Log F = 0.8107 + 1.1793 Logw).

7. The overall female to male ratio in the commercial catches is not significantly different from 1:1 though there is a preponderance of female sex during most of the months. Sex-ratio variation from 1:1 is statistically significant in various size groups (at 10 mm intervals), with male dominating upto 170 mm and female dominating above this size.

8. The internal ovarian structure reveals that it is of cystovarian type. The ovarian wall is made up of an outer layer, *tunica albuginea* and an inner layer, germinal epithelium. The latter is thrown into several folds, known as ovigerous lamellae, inside the ovocoel which harbour oocytes in different stage of maturity.

9. The ovarian follicle consists of the oocyte and its various outer covering membranes. The acellular layer lying just outside the oocyte is the zona radiata, which exhibit a bipartite structure in advanced stages of maturity. Follicular epithelium consisting of granulosa cells lies outside the zona radiata and a less prominent connective tissue layer, theca, forms the outermost layer of the ovarian follicle.

10. Two phases in the growth of oocytes, namely previtellogenesis and vitellogensis have been observed. Based on the size, amount and distribution of various cell inclusions, the oocyte development is classified into 7 stages, namely primary oocyte stage, vacuolated oocyte stage, primary yolk granule oocyte stage, secondary yolk granule oocyte stage, tertiary yolk granule oocyte stage, hyaline oocyte stage and atresia. Atresia has further been classified into 4 stages, namely a-, b-, c- and d-atresia.

11. The observation that *S.sihama* is a multiple spawning fish as revealed by conventional ova diameter frequency studies, has been confirmed by analysing the mean percentage frequency distribution of oocyte classes in the ovarian histological preparations. Study of the relative volume of various oocyte classes in the ovaries indicates that yolk granule oocytes and hyaline oocytes have more relative volume than the others in the mature and ripe ovaries.

12. Histological study of the testes of *S. sihama* shows that, they belong to the 'lobular type', since the body of the testis is made up of a number of seminiferous lobules of different size. The testis is also of the 'unrestricted type' since each seminiferous lobule is made up of

seminiferous cysts containing different spermatogenic cells. The branching pattern of vasa efferentia and the arrangement of lobules further indicate that the testis is of 'radial type'. Externally the lobules are lined by spindle shaped boundary cells. The interlobular space is made up of connective tissue with a few Leydig cells and blood vessels.

13. Based on the size of cells, the nuclear characteristics and the cytoplasmic morphology, the spermatogenic cells have been classified into 6 cell types, namely primordial germ cells, spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoan. Occurrence of these spermatogenic cells in each maturity stage of testis is discussed in detail.

14. Estimation of carbohydrates (total carbohydrates, glycogen and glucose), protein, lipid and cholesterol in muscle, liver, gonads and blood plasma, during different maturity stages reveals that there is depletion of these constituents from the somatic tissues during maturation. The somatic tissue depletion of the energy sources appears to be partly due to their translocation to the gonads for the synthetic activity during gametogenesis and partly for meeting the energy demand of the fish just prior to spawning.

15. The biochemical variations are more prominent in female than in male during gonadal maturation. Further, sex-related differences in the biochemical composition are more perceptible during the final stages of maturation, with the female showing relatively more depletion of energy than male with advancement of maturation.

16. The distribution of the different groups of carbohydrates, proteins, lipids and nucleic acids in the oocytes during different stages of maturation is

studied by the qualitative histochemical tests. Follicular epithelium is found to contain traces of general proteins, amino groups, S-S groups, tyrosine and tryptophan and moderate quantities of RNA. Carbohydrates are found to be more abundant in the zona radiata externa (ZRE) than in the zona radiata interna (ZRI). While ZRE contains lipids, ZRI does not. Acid mucopolysaccharides are absent in the oocytes of *S. sihama*

17. Yolk vesicles, which make their appearance in the cortical cytoplasm of the primary yolk granule oocytes, contain carbohydrates and -SH groups; lipids are not detected. Of the two yolk bodies, lipid yolk makes its appearance first, in the peri-nuclear area of the vacuolated oocytes. These oil droplets are found to contain neutral and phospholipids. Protein yolk initially accumulates in the cortical region of the oocytes and gradually extends centripetally to fill the ooplasm. Histochemical staining of protein yolk reveals the presence of general proteins, amino groups, S-S groups, tyrosine and tryptophan; -SH groups are detected only in traces, while lipids are absent.

18. The oocyte nucleus shows the presence of DNA only in early maturity stages. Similarly, nuclear RNA content also shows decrease with advancement of maturation. Moderately sudanophilic intranuclear lipid bodies are noticed in the primary oocytes and vacuolated oocytes. Nucleolus is found rich in RNA and proteins. 'Nucleolar extrusions'. have been observed in the vacuolated oocytes.

19. Results of some preliminary experiments on induced maturation and spawning of *S. sihama* collected from Cochin backwaters reveal that

ovarian maturation can be induced by major carp pituitary gland extract (60, 100, 120 and 150 mg/Kg body weight), HCG (3000 and 5000 IU/Kg body weight) or a combination of both (60 mg carp pituitary gland extract + 1000 IU HCG per Kg body weight).

20. Female was found to spawn after receiving either 150 mg/Kg body weight of pituitary gland extract or 3000 IU/Kg body weight of HCG. Males in 'non-seminating' condition developed to oozing stage on receiving either 280 mg/Kg body weight of pituitary gland extract or 1000 and 2000 IU/Kg body weight of HCG.

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